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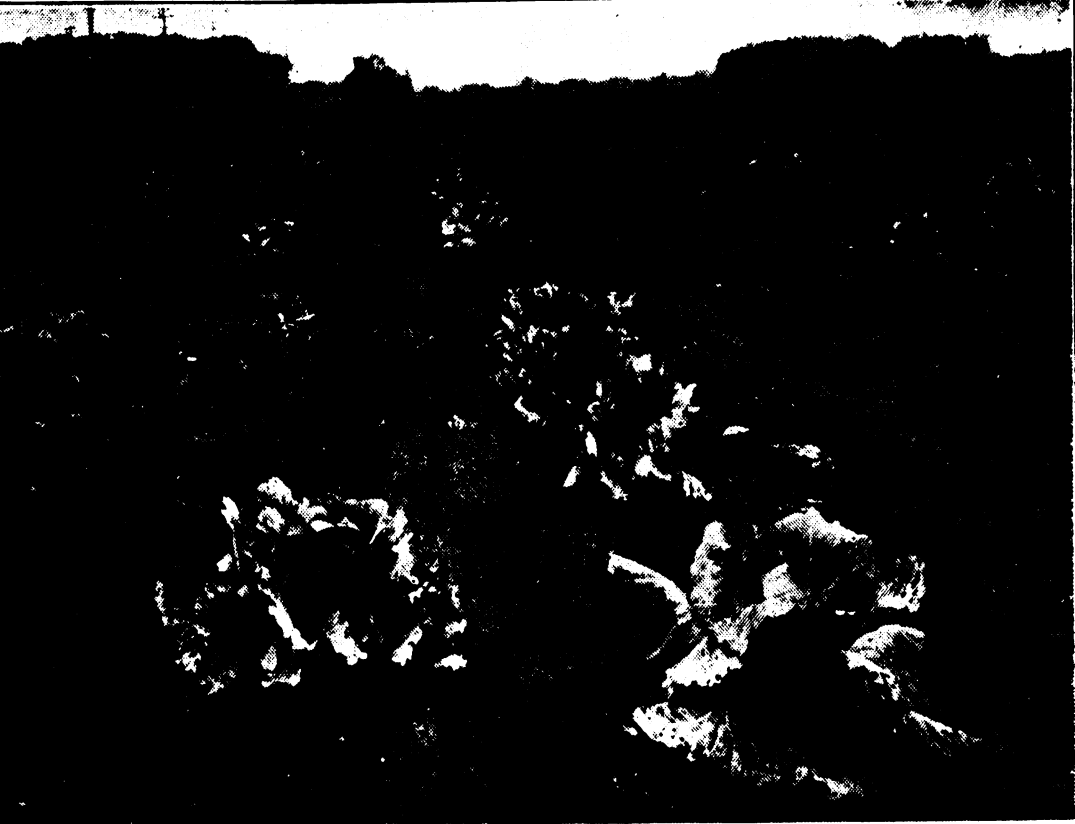
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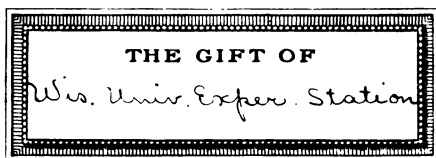
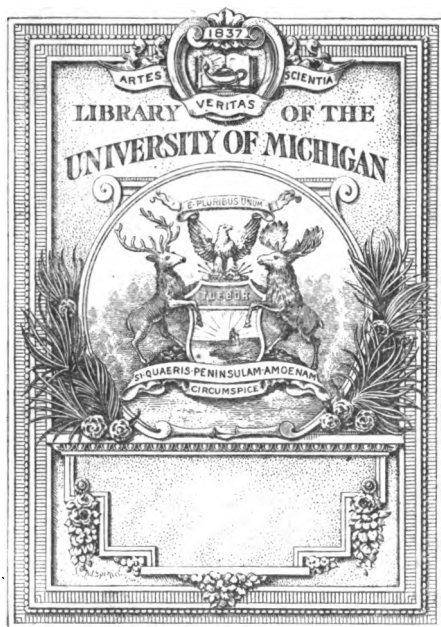
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*Annual report of the Agricultural  
experiment Station of the ...*

University of Wisconsin--Madison.  
Agricultural Experiment Station





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LIVESTOCK SPECIAL, IN CHARGE OF INSTRUCTORS IN THE DEPARTMENT OF ANIMAL HUSBANDRY  
One of the Most Vital Features of University Extension Work

TWENTY-NINTH ANNUAL REPORT

OF THE

Agricultural Experiment Station

OF

THE UNIVERSITY OF WISCONSIN

*For the year ending June 30, 1912*

CONTAINING RESEARCH BULLETINS NOS. 19 TO 24 INCLUSIVE

*Issued 1912-13*



MADISON, WIS.  
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1912



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# The University of Wisconsin

## Agricultural Experiment Station

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**PART I**

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**REPORT OF THE DIRECTOR**

**1912**



**PART I**

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**REPORT OF THE DIRECTOR**

**1912**



# REPORT OF THE DIRECTOR

1911—1912

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H. L. RUSSELL

Twenty-five years ago, the Federal Congress passed the Hatch Act, introduced by Representative Hatch of Missouri, founding the system of agricultural experiment stations. These institutions were for the "purpose of acquiring and diffusing among the people of the United States useful and practical information on subjects connected with agriculture, and to promote scientific investigation and experiment respecting the principles and applications of agricultural science."

The beneficial results arising from this act have become better known as time has gone on. Twenty-five years ago, the practical farmer was very skeptical as to what science could do to aid him in his work. The early station leaders had an untried field before them, and a critical public with which to deal. With all of the apathy and opposition that obtained among the very class which this work was designed to help, the wonder is that Congress had the foresight to enact into law a measure of this character.

The original Hatch Act, appropriating to each state \$15,000 annually, was supplemented in 1906 by the Adams Act\* which added ultimately another \$15,000, making the federal grant to each state \$30,000 in all. The expectation was that as needs required this nucleus would be added to by the state itself. In this state, as in many others, this moral obligation has been met in a generous way.

Experimental work in this period of twenty-five years has undergone much transformation. The simple, evident problems

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\*Hon. H. C. Adams, Member of Congress from this state and district, was the author of this supplementary Act.

that were comparatively easy of solution have now in large measure been solved. The problems of the future are necessarily more fundamental and oftentimes may not seem to have any direct practical bearing, but no one can foretell what highly beneficial results may be developed from the most profound researches. The American experiment station movement has set a standard that has served more or less as a model throughout the world. To-day, the interest in the work of these organizations is widespread, and by no means confined to the farmers themselves. The press and the general public now show a keen appreciation of things agricultural—a radical change from the apathy and even opposition of the earlier days.

The successful and efficient experiment station cannot remain content with mere investigation in the laboratory. The principles worked out here in detail must be tried out in the crucible of field experience to make sure that wrong conclusions are not put forth on the basis of inadequate data. The results of the laboratory must be extended to the farmers of the state.

## RESEARCH WORK OF THE EXPERIMENT STATION

The synopsis here presented records the more prominent lines of investigation that have been in progress during the fiscal year ending June 30, 1912. The completed results are published in bulletins from time to time, but these form at any time only a fraction of the entire group of problems that are under investigation. There is always much work that is only partially completed, but not infrequently, tentative conclusions concerning these questions can be presented that may be of real value to the public. The summary here recorded may therefore be considered in the light of a progress report of the activities of the past year.

### PEA BLIGHT

One of the growing industries in the state is the pea canning business. The soil and climatic conditions in the shore regions of our great lakes, as well as some of the centrally located counties of the state, seem to be preeminently well suited to pea culture. Forty-five canning companies are reported this

year as growing 35,000 acres, while approximately 15,000 acres more are devoted to seed production.

Within recent years trouble has, however, developed in certain sections of the state, and the profitableness of the business has been in some cases seriously impaired. A year ago the ravages due to a disease known as pea blight were so severe in some sections as to cause almost total loss. This year 12 companies that were in operation in 1909 are out of business and others have been forced to turn from peas to vegetables.

Realizing the menace to their industry, the Pea Canners' Association last year importuned the Station for aid, but as funds had already been allotted, it was impossible to provide for this



FIGURE 1. PEA BLIGHT CAN BE CONTROLLED BY SCIENTIFIC METHODS  
Spraying with Bordeaux mixture helps to control this fungus disease, in this trial more than doubling the yield of peas.

work. The Canners' Association thereupon subscribed a fund sufficient to permit research work to be undertaken, and Prof. L. R. Jones and Mr. R. E. Vaughan have been studying the problem, Mr. Vaughan's expenses being paid from this fund.

Several parasitic fungi have been investigated and the life history of two types sufficiently studied to make possible the trial of a rational system of prevention. The custom of growing the crop on the same areas without rotation (which practice has been generally followed where the canning company owns the land) affords most excellent conditions for the development of the disease, as the winter spores of the fungus mature on the pea stubble, and so infect the young plantlets as the seed germinates the next spring. One of the fungi lives over in the seed itself, and hence can be successfully prevented only by the use of healthy seed.

Field experiments were conducted in which pea straw as well as pea silage were plowed under. These showed a marked



increase in amount of diseased tissue, and a reduced yield of pods, in comparison with check plots. Spraying with Bordeaux mixture helps to control the disease. The results of the year already point out quite clearly the course which the growers will have to follow in order to hold these diseases in check.

#### CABBAGE DISEASES

Many of the cabbage growers in Racine and Kenosha counties have been driven out of business, so far as cabbage culture is concerned, through the ravages of certain fungus diseases affecting this plant. Prof. L. R. Jones, continuing his studies on these different diseases, has found that various commercial fertilizers, as well as soil disinfectants, are wholly useless as preventive agents for the control of this disease in infected soil. For several seasons he has turned his attention chiefly toward the breeding of resistant strains and this year is able to report most substantial progress in this direction. In fields planted with commercial varieties in 1910, where the disease caused almost an entire loss, the few naturally resistant heads were selected, and seed raised therefrom in 1911. While commercial seed planted on infected fields gave this year only 21% of living plants, the cabbage grown from the "resistant" seed developed 86% live plants, over half of which formed heads. Seed produced from the best head gave 93% of properly matured heads. Though the disease was not so destructive this year as it was in 1911, these results show the influence of careful selection and indicate clearly the great possibilities that lie in the use of home grown seed of disease resistant strains adapted to local conditions.

#### TOBACCO DISEASES

Continuing his study of tobacco diseases, Mr. Johnson of the Horticultural department has found that the strength of formalin solution ordinarily recommended as a soil drench to prevent damping off in the seed bed, only checks the development of the damping off fungi for a short time. However, when a stronger solution, one part formalin to 50 parts of water, is used, the fungi are killed. Steam sterilization has proven very satisfactory, not only preventing damping off, but also killing weeds and greatly increasing the rate of growth and the gen-

eral vigor of the plants, as is shown in Figure 3. A root rot of tobacco due to the fungus, *Thielavia basicola*, which is a serious disease in the eastern states, during the past year caused

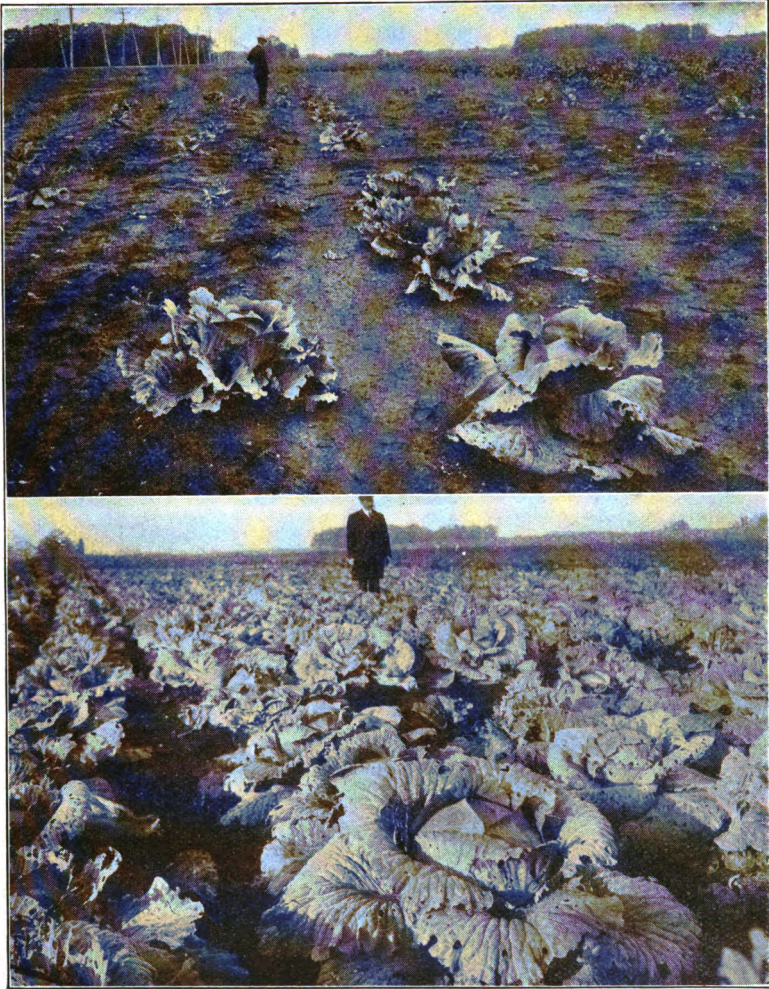


FIGURE 2. USE OF "RESISTANT" CABBAGE SEED SAVES CROP

- (A) "Cabbage-sick" field (Racine) in 1911, plants nearly all destroyed.  
(B) Same field in 1912, plants grown from "resistant" seed.

considerable injury in this state for the first time. Our growers should at once adopt the measures for its control which have been worked out in the East. Observations on the fungi which cause diseases in the curing shed have been continued, and

a number of different fungi have been found to be capable of producing shed troubles under certain conditions.

The "black rot" of tobacco, a disease occurring during fermentation, has been conclusively shown to be due to a fungus, *Sterigmatocystis nigra*. The determination of the cause of this trouble has opened up the possibility of several different methods for its prevention, especially the control of the moisture content of the leaf and of the temperature of fermentation.



FIGURE 3. EFFECT OF STEAM STERILIZATION OF TOBACCO SEED BEDS

Plants on sterilized soil show increased growth and freedom from weeds. Right, not sterilized, but weeded; left, sterilized, and not weeded.

#### PLANT DISEASE SURVEY

While the year on the whole has favored crop growth, Professor Jones reports that certain plant diseases have likewise been favored by weather conditions. With potatoes in general there has been this year but little disease. The moist, cool condition of late summer, however, led to a recurrence of the late blight, the most destructive potato disease, which has been held in abeyance the two preceding years by the hot, dry summers. Potato rot also occurred in certain localities. While late blight can be controlled by spraying, it is important where the disease occurs to bear in mind that it is perpetuated in seed tubers from diseased fields, unless precautions are taken in seed selection.

The diseases of cabbage this year have proven quite destructive, black leg, black rot and yellows appearing in a number of sections. The necessary precautionary measures to observe in cabbage culture are seed disinfection, a clean, non-diseased seed



bed, sanitary practices with reference to culture, selection of disease-resistant strains, raising one's own seed, and proper crop rotation as well.

Pea diseases this year have not been as serious as last year, owing to the cool, moist weather, favoring very rapid and satisfactory growth. Much disease, however, was found in the incipient stages.

Orchards, especially apples and pears, suffered last year from winter injury occasioned by the severe climatic conditions of the previous winter. This "winter killing" should not be confused with fire blight, which is a specific, communicable, bacterial disease. Apple rust was destructive with certain varieties.

The barley blights were not as destructive this year as the year before, yet were widely distributed over the state and in certain cases caused as high as 40% loss.

Oat smut, which has heretofore been held in abeyance for a number of years through the widespread introduction of the formalin treatment, is now beginning to assume importance, due to neglect of this precautionary treatment. Some fields were found this year in which over 40% of an otherwise good stand was destroyed by the disease.

It is generally recognized that one of the most important ways of conserving agricultural resources is to give due attention to the various plant diseases and pests and their control. This is true not only for the ordinary orchard and garden but is especially true where field crops are concerned.

#### BARLEY DISEASES

Further investigations on the leaf blight of barley, conducted by Mr. A. G. Johnson of the Plant Pathology department, show that there

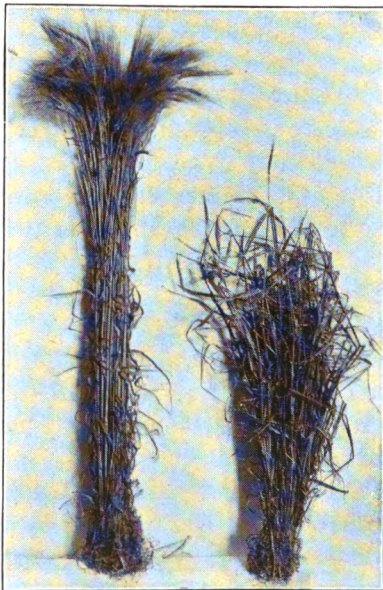


FIGURE 4. BARLEY "LEAF STRIPE"  
One hundred healthy plants on left; same number of diseased plants on right.

are three distinct species of fungi concerned, each of which is capable of producing a specific disease. Apparently the most severe trouble is what has been designated as "leaf stripe" disease, in which the disease manifests itself as light colored stripes in the leaves, which later turn gray and then brown. As the malady progresses, the entire plant collapses. The other two diseases, causing leaf blotch, produce a somewhat similar appearance. All three troubles have been found widely distributed throughout the state and are doubtless as important as stem rust, and may become even more destructive when weather conditions favor. A hopeful aspect of the situation, however, is the possibility of control. Field experiments this summer have shown that the formalin treatment of seed, as is ordinarily practiced for the covered smut of barley and for oat smut, is effective in controlling the leaf stripe, but is less effective against the leaf blotch disease.

#### INSECT PEST SURVEY

Almost perfect crops of fruit were harvested in Wisconsin in 1911 without spraying, due to the nearly complete loss of fruit the previous season caused by the late spring freeze, and the consequent depletion of the insect pests through starvation. But Professor Sanders of the department of Economic Entomology in the last annual report especially emphasized the necessity of close control for the season of 1912, since the great reduction of insect pests had also lessened their natural parasites, and unusually rapid multiplication of the insects would likely occur for several years until the parasites again attained their normal prevalence. This prediction has been only too well verified during the past season.

While the codling moth, the larvae of which are the common "apple worms", was almost wholly eliminated in some regions in 1911, this year the pest was very abundant and caused a large loss of fruit where no control sprays were used, or where sprays were improperly applied. It is estimated that this one pest causes a loss of 15 to 20 millions of dollars each year in the United States, and these losses will continue until fruit growers generally adopt those spray control methods which have been determined by entomologists to be satisfactory, such as spraying with paris green or arsenate of lead.

An unusually severe outbreak of white grubs, the larvae of the June beetle, caused great damage to corn and some other hill crops this year in the southwestern quarter of the state, and in other scattered localities over the entire state more or less injury was apparent. No known remedies for the control of white grubs and wire worms are satisfactory, but preventive measures, consisting of late fall plowing, and discing or harrowing, have proven beneficial in destroying the hibernating forms in the ground. Complete summer fallowing of infested fields will starve the white grubs, through preventing the growth of any plant life. Hogs will also do very thorough work by rooting up the soil and devouring them.

Local outbreaks of several species of cutworms, including the so-called army worm, occurred in the early summer. These can be readily controlled with poison bran mash made as follows: Thoroughly mix one pound of paris green in 30 or 40 pounds of dry bran; moisten slightly with cheap, thin syrup until the mass will hold together well when pressed in the hand. Distribute this poisoned bait about plants to be protected, or sow it broadcast in gardens or fields about sunset, and exclude all poultry until after a rain to avoid danger of poisoning.

The corn ear worm appeared in injurious numbers in some localities. Although this is a member of the cutworm group, the poison bran mash is not effective as a control. Fall plowing and thorough cultivation is our best control in this case.

Strawberry leaf rollers nearly ruined some plantations through lack of the owner's care, when a few sprays of arsenate of lead—4 pounds to 50 gallons of water—would have killed off the larvae. This is our most important strawberry pest in the state, with the possible exception of the white grub.

Leaf mining larvae were unusually abundant on various plants and trees, but judging from the large proportion of these insects infested with parasites, the injuries will probably decrease next year. A new use for nicotine solutions in the control of these larvae was determined, and such good results were obtained experimentally that the method will be tried out further in the field.

Although locusts or grasshoppers were unusually destructive in some sections in 1911, these pests were so greatly reduced in number the past season that serious injury was reported in but few widely separated localities. These changed conditions

were due to the many natural enemies of the grasshoppers, including fungus diseases, internal worm parasites, predaceous mites, and the larvae of the blister beetle.

Professor Sanders especially urges that fall plowing be done wherever and whenever possible in preparing land for planting, because only by following this practice can some of our common field pests be controlled and kept below the danger point.

#### CRANBERRY INSECTS

Mr. Malde has continued the observations on cranberry insects begun several years ago, in cooperation with the U. S. Bureau of Entomology. He reports a scarcity of insect pests this year, which may be attributed to the heavy fall rains that occurred shortly after the cocoons were formed and also this spring when the insects were passing through the changes which take place before the adult stage is reached. The severe winter and scarcity of snow also caused deep freezing.

The tip worm, however, was abundant this year. Damage from the fruit worm, which occasioned much loss in 1911, was slight this year, owing to its comparatively late appearance. In Price county 75 miles from any cultivated bog, and also in Washburn county, on wild marshes, millers of the yellow head vine worm were found late in May feeding on leather leaf and sage bush (*Andromeda*). This indicates that these insects, and also the fruit worm, which was found late in August at work on wild marshes near Phillips and Merrill, are native pests in the state, and only periodically become plentiful enough to be noticed readily.

#### AVIAN TUBERCULOSIS

For several years Professor Hastings of the Agricultural Bacteriology department has been studying the question of tuberculosis of fowls. Reference to the literature on this subject indicates that this disease has not been recognized here in Wisconsin as one of much economic importance, but from the data accumulated by Professor Halpin of the Poultry department, it is evident that it is quite widely spread throughout our state. With the increasing importance which is now being given to the poultry industry, it is decidedly important that

those interested in poultry husbandry should be cognizant of the situation.

Avian tuberculosis, unlike the mammalian form of the disease, is primarily an affection of the liver and spleen. The experiments indicate that the disease is contracted mainly through feeding, that it can be transmitted from fowls to such mammals as swine, and that close contact of healthy and diseased birds will transmit the infection. The main danger, therefore, as with the disease in cattle, is in introducing diseased stock into the flock. Unfortunately, the owner cannot safeguard the condition of his flock by applying the tuberculin test to his purchases, as he can with cattle.

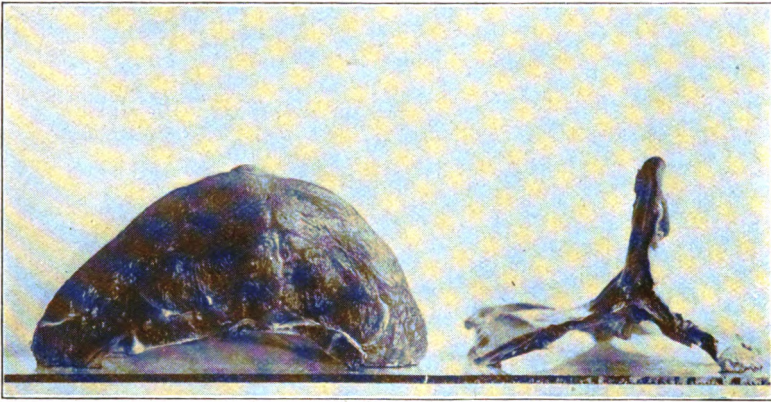


FIGURE 5. TUBERCULOSIS OF FOWLS

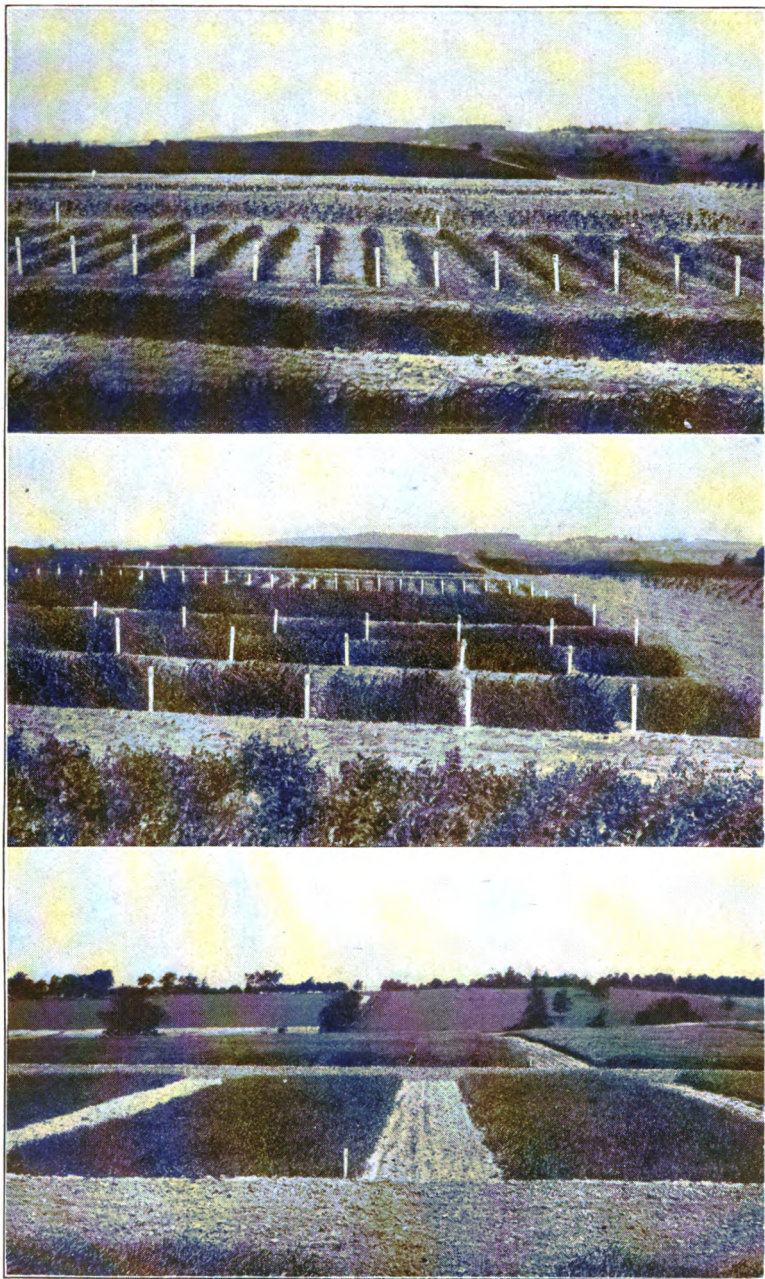
"Going light," or wasting away, characterizes tuberculosis in chickens. On left, cross-section of breast from healthy fowl; on right, withered breast of tubercular fowl.

#### IMPROVED GRAINS AND FORAGE CROPS

On the Hill farm at the Station, the Agronomy department had under cultivation this year 90 comparative test plots and 183 centgener breeding plots planted to oats, millet, and wheat. Eleven varieties of pedigree barleys were in the advanced breeding plots; 8 varieties of oats were in the field areas to determine yields and other characteristics.

Pedigree rye yielded this season at the Station, 49 to 54 bushels per acre by weight on land which had been previously in alfalfa. This variety of rye has undergone careful selection by the Agronomy department for a number of years, and has now been disseminated through the state so there are about 1,200 centers where pedigree seed may be obtained.





**FIGURE 6. GRAIN BREEDING PLOTS AT THE HILL FARM**

Starting with the head-to-the-row test plots (a), the next year the best hundred plants are developed in centigener plots (b), after which increase plots (c) are grown.

The pedigree barleys heretofore developed have been under test a number of years. The breeding plots on the Agronomy fields this year yielded from 44 to 57 bushels per acre. These varieties have now been in the hands of the members of the Wisconsin Experiment Association for five years. In over 1000 reports collected during this time, the pedigree varieties showed an average yield of 4.9 bushels more than the best competing varieties. An important feature of these pedigree barleys is the fact that the kernels are of uniform size, vigor, and quality, thus on account of uniformity in germination being far superior to the ordinary mixed strains for malting purposes.

The test plots of oats yielded from 68 to 110 bushels per acre, which is 20 bushels more than ever before grown on our increase plots under ordinary farm conditions. An interesting report was received by Professor Moore from one correspondent who planted one bushel of Wisconsin Pedigree No. 1 oats rather thinly, making it cover nearly an acre. He reported a 90 bushel crop from such seeding. This seems to indicate most excellent stooling properties in this strain.

Considerable work has also been done in crossing barleys, especially the bearded pedigree, with the beardless barley. About one hundred crosses were made on wheats and fifty on oats, from which future selections will be made.

On the county demonstration station at Superior, excellent results were secured this year in growing flax. A good yield was produced on planting made even as late as the first of June. This crop seems well adapted to new breaking, especially since virgin soil is always free from the "wilt", and therefore insures good yields. Where land is cleared in the spring, a cash money crop can be secured the first season. Sixteen to 18 bushels of flax have been secured on the red clay for the past three years. As there are linseed mills for the grinding of flaxseed, and also elevators for the handling of the same at both Superior and Duluth, it is possible to dispose of the product advantageously in this portion of the state. Climatic conditions are apparently very suitable, as flax is a cool weather crop.

Good results have also been secured in breeding and selection work at the branch stations. Professor Delwiche of the Agronomy department who has charge of all the work of that department in upper Wisconsin, reports on the sandy soil at the Spooner station a yield of 73 bushels per acre of Wisconsin

No. 8 corn, which has been especially acclimated for the northern part of the state. This corn matured fully in this section which was once considered far outside the corn belt. At the Ashland branch station, on the heavy red clay, Pedigree No. 8 wheat, a strain of Kharkoff winter wheat, yielded 35 bushels per acre.

#### ALFALFA CULTURE

As alfalfa seems destined to become one of our most important and valuable crops, the Agronomy department is conducting extensive trials on various phases of its culture. These include studies on the quality and hardiness of alfalfa raised in Wisconsin from southern and northern grown seed, from seed produced on irrigated and dry land, and from the much advertised, imported Grimm seed. The results so far secured indicate that the variety sown is not so important as the vitality of the seed.

While the common and usually recommended rate of seeding is 20 pounds per acre, the statement is now being made by some that good stands of alfalfa can be secured in Wisconsin by seeding with as little as 8 pounds. Tests are in progress here at the Station, also in cooperation with 250 members of the Alfalfa Order of the Experiment Association, to determine the best rate of seeding for this climate, as well as the best time for summer and fall seeding.

The experimental work in progress at each of the branch stations includes tests of the value of seeding with and without a nurse crop, liming the soil, inoculation with soil and commercial cultures, and the application of manure. At the Spooner branch station, inoculation with soil gave better results than the use of a commercial culture.

On the Kennan clay loam soil at Conrath, liming was of benefit in securing a good stand of alfalfa. Here, also, inoculation with soil proved superior to either no inoculation or inoculation with a commercial culture.

#### CRANBERRY CULTURE

The Station bog at Cranmoor in charge of Superintendent Malde again demonstrated this year the value of intensive scientific methods over the more extensive methods of culture. Insur-

ance against injury from summer frosts is practically secured by the "sanded" bog method. In June on five consecutive nights, from the sixth to the tenth, all of the surrounding bogs of the old type had to be flooded for protection. Where the bog was not so handled considerable loss occurred. On the station bog no flooding was done from spring till fall but complete immunity from summer frosts obtained.

The application of commercial fertilizers made several years ago to certain of our station plots continues to show excellent results. Phosphate and nitrate plots yielded at the rate of 150 bbls. per acre while the unfertilized plot produced 91 bbls. per acre. Iron sulfate solution has been used this year with success as a weed killer on new plantings and young vines. Two applications were sufficient to hold many of the troublesome weeds in check and retard grass growth.

The crop this year was good throughout the state, although some loss was sustained by frost in June, especially in the Berlin district, and from flooding in September. The station had the largest crop it has had for years. The high rain fall of the season has, however, injured materially the keeping quality of the fruit.

#### NEW APPLES FOR WISCONSIN

For many years apple seedlings have been grown and tested by the Horticultural department in an attempt to secure better varieties for use in the state. As the seedlings have borne fruit, only those of promise have been kept. Prof. J. G. Moore now reports that three seedlings seem to be worthy of testing out in the field. One of these, a seedling of the McMahon, has been highly spoken of by several fruit growers who have seen it on exhibition. A seedling of the Walbridge gives evidence of being a great improvement over the parent, and a Fameuse seedling, of entirely different type from the parent, seems of merit for Wisconsin conditions.

In addition to the testing of seedlings, the newer varieties of apples have been thoroughly tried. Of those under trial, the Hydes King and Garfield seem suited to Wisconsin conditions and are therefore being propagated for future dissemination.

### HEMP AS A WEED ERADICATOR AND MONEY CROP

Two years ago Professor Norgord of the Agronomy department was successful in eradicating quack grass and Canada thistles from a field on the state prison farm at Waupun by the growth of hemp, preceded by fallowing the previous summer. Not only were these weeds eradicated, but a yield of fiber valued at \$118 per acre was secured. As a result of this success, the past two years 200 acres of hemp have been grown by farmers around Waupun and Fox Lake, a large part being placed on thistle and quack infested lands. The 1911 crop



FIGURE 7. SHOOKED HEMP READY TO "BREAK"

A crop yielding \$80 to \$100 per acre, that will kill out Canada thistles and quack grass is worth considering if your land is infested with these noxious weeds.

yielded from 800 to 1200 pounds of fiber per acre, the long fiber being sold this season for seven, and the short fiber, or tow, for five and one-half cents per pound.

In certain instances where an exceedingly tough quack grass sod was not worked the previous season to kill some of the grass, the hemp was crowded out. Lack of fertility had the same effect. To be sure of getting results, the land should be fallowed during the latter half of the previous year, manured well, and, if possible, plowed just before sowing hemp in the spring.

Trials on upland indicate that hemp cannot be profitably grown on any but the best lands. Aside from a lack of fertility, the greatest difficulty lies in the danger of the soil packing, particularly if this is followed by hot weather. To prevent

this trouble the hemp may be (1) placed on corn land which was in sod the previous year; (2) grown on sod land, or (3) manured with plenty of strawy manure and then top dressed, if possible, with manure not containing too much straw, and thoroughly disced into the soil. Experiments conducted on the Horicon marsh indicate that peaty land will produce a large crop of hemp with good fiber.



FIGURE 8. HEMP KILLS CANADA THISTLES

On left, dead Canada thistle plants with rotten underground rootstocks killed by the dense growth of hemp. On right, vigorous, healthy thistles from margin of same field.

#### SEX-LIMITED INHERITANCE IN THE DOMESTIC PIGEON

In order to study the principles governing the inheritance of various characteristics, Professor Cole of the Experimental Breeding department is conducting experiments with small animals, such as pigeons, rabbits, rats, and mice. One of the interesting points already brought out is the relation of sex to the inheritance of color in domestic pigeons. Professor Cole had already shown in earlier work with these birds that dun, yellow, and silver are dilute conditions of the so-called intense colors—black, red, and blue, respectively. In the case of cer-



tain crosses made last year in which the male parent was a dilute (yellow or dun) and the female a black, both black and dun offspring were produced. As the young birds matured, it became evident that all the blacks were males and all the duns were females. Further investigation showed that this was a typical case of sex-limited inheritance, in which in stock bred true for a certain character, one sex (the female in pigeons and poultry) never breeds true to type. These discoveries furnish a satisfactory explanation of certain formerly inexplicable results secured in breeding, where birds bred supposedly "pure" for color have not bred true. Examples of sex-limited inheritance have been found in the case of fowls by other investigators in the inheritance of barred feathers, as in the Plymouth Rock fowl, and more recently, in the inheritance of egg production. Should the same rule prove to hold generally for other domestic animals, it will be a matter of much importance to the practical breeder.

#### SOIL MANAGEMENT

A considerable part of the research work of the Soils department on the management of different soil types is necessarily carried on in various portions of the state.

Studies on the sandy types are in progress at the branch station at Spooner, and also at Crivitz, while the department has entire control of an experimental field on the much depleted sands near Sparta.

On these poor sandy soils Professor Whitson finds, when the growing conditions are so adverse as to make it impossible to secure a catch of clover with a nurse crop, that better results are obtained where the clover is seeded alone. Deep seeding and heavy rolling after seeding, also proved beneficial.

The work in progress at Sparta is on extremely sandy soil that had been so exhausted by thirty years of cropping that six acres of timothy produced only one small load of hay. While only meager returns were secured during the first three or four years, the past two years have shown marked improvement. The results now seem to justify the conclusion that these level sandy soils even of such coarse texture and so low in organic content that they have been left largely without agricultural use may be farmed with fair success if careful use is made of

suitable legumes, supplemented by moderate applications of essential mineral fertilizers.

It has been found that when moderate amounts of potash and phosphorus are applied, good crops of serradella, yellow lupine, and cowpeas may be grown on these very poor, slightly acid sandy soils without liming, while with the same treatment, medium red and mammoth clover fail entirely. However, in 1911, by the application of lime, in addition to fertilizers containing phosphorus and potash, a catch of clover was secured which this year yielded 2.58 tons of hay per acre at the first cutting. Corn and potatoes grown on soil where these legumes have been used as fertilizers have yielded encouraging, though small crops.

Experiments at Crivitz in Marinette County on sandy soils similar in texture and mode of origin to the Sparta field, but in a virgin condition, have yielded more encouraging results, and demonstrate the necessity of adopting methods at the beginning for the maintenance of the initial fertility of such soils. In 1911, plots of this sandy acid soil were inoculated and seeded to alfalfa, with and without the application of lime. This year the limed plot gave 43.3 per cent increase in yield over the unlimed plot.

The change from the old temporary substation at Ashland to the new permanent branch station at Ashland Junction has interrupted the soil work on the heavy clays. Professor Whitson has secured data this season from the old Ashland station confirming the need of phosphorus treatment on that type of soil. An increase of 10% in potatoes and nearly 12.5% in clover hay was secured by application of rock phosphate.

#### TILE DRAINAGE

Professor Jones of the Soils department is continually accumulating data on the efficiency of drainage systems. Where the subsoil is of clay he finds that mains with a capacity which will remove one-quarter of an inch of rainfall per twenty-four hours are sufficient. In cases where there were springs to be drained, however, the maximum discharge has been as high as nine-tenths of an inch in twenty-four hours. This emphasizes the fact that in determining the amount and size of tile it is necessary to consider fully the peculiarities of the area, so as to have adequate discharge. Professor Jones



has in progress studies on peat drainage in Portage county where the underlying sand is used as a basis for under-drainage.

### STATE SOIL SURVEY

Work has progressed rapidly this past season on the continuance of the State Soil Survey, which has been carried on co-operatively by the Soils department of this College, the State Geological and Natural History Survey, and the Bureau of Soils of the United States Department of Agriculture.

Two types of surveys are in progress in connection with this work: first, a reconnaissance or preliminary survey, in which it is planned to map in a general way the distinct types of soil in as much detail as is practicable in the present condition of the development of these regions; second, the detailed surveys which are being carried out in the older settled regions of the state, as well as in those portions of the north where intensive development is in progress, such as the fruit region of the Bayfield district. These involve a much more complete and detailed examination of soil conditions.

The field data collected under these conditions are designed to give all necessary information relative to the character of the soil. Chemical and physical examinations are made on type soils in the laboratory.

Reconnaissance work in the northern section of the state has included this season an examination of the soils in Douglas, Bayfield, Burnett, Washburn, Sawyer, Ashland, Forest, and Florence counties. Also, in connection with the State Forestry department, examinations of soils have been made in part of the state forest reserves, to determine the agricultural value of certain lands.

The field work of detailed soil surveys was completed in 1911 in Fond du Lac, Juneau, La Crosse, Kewaunee, and Columbia counties, and in 1912 in Jefferson county, while in Bayfield and Dane counties, similar surveys have been started.

In all eighty-five different types of soils, which have been classified into seventeen series, have been described in the detailed work. The area already covered in the survey is shown in Figure 9, the year in which the survey was completed in each county being indicated. Much time must necessarily elapse after the completion of the field work, before the analyses of the soil

samples are completed, and reports are published. As the reports for the various counties are brought out, the fact will be advertised in the local papers. Reports are now available for only the western area mapped by the Geological and Natural History Survey and for the reconnaissance survey of Marinette county.

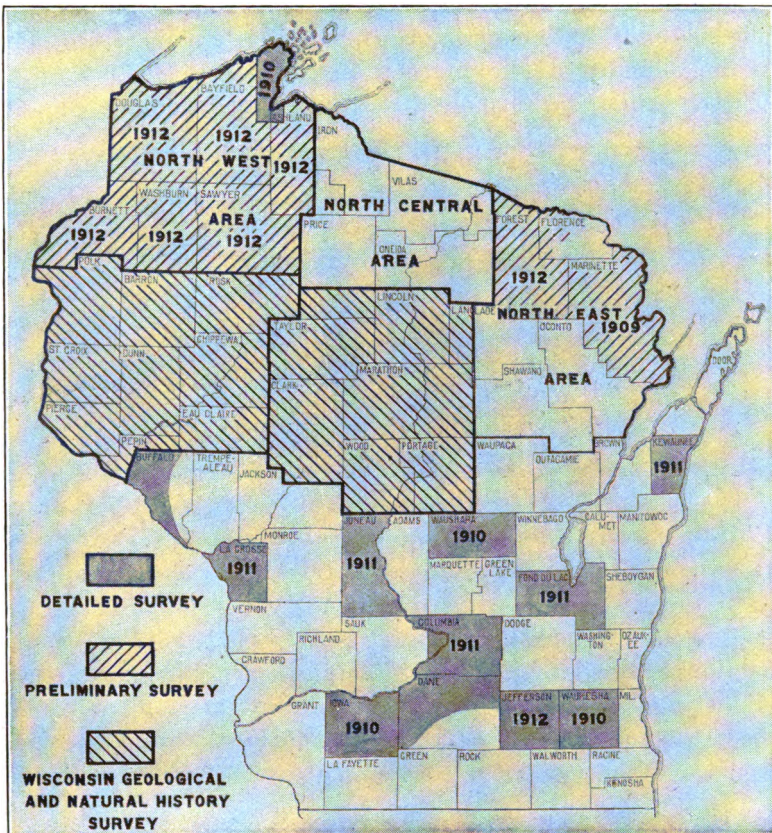


FIGURE 9. PRESENT STATUS OF SOIL SURVEY

A knowledge of what the soils are in any region is a prerequisite to any suggestions as to the best methods of management.

### CONDITION OF PHOSPHORUS IN SOIL AND AVAILABILITY OF VARIOUS PHOSPHATES

Many Wisconsin soils which are high in total phosphorus, are, however, deficient in available phosphorus as measured by

solubility in dilute acids, and will generally respond to phosphorus fertilizers. Professor Peterson of the Soils department has accordingly been studying the condition of phosphorus in typical soils, especially peat. By oxidation of the soil with varying amounts of hydrogen peroxide and extraction with dilute acid, he finds that as the organic matter is destroyed the amount of soluble phosphorus is increased. There is a corresponding increase in the solubility of iron and aluminum, but the solubility of calcium and manganese is not increased, nor is the solubility of the nitrogen increased in a constant ratio. These results tend to show that the increased solubility of the

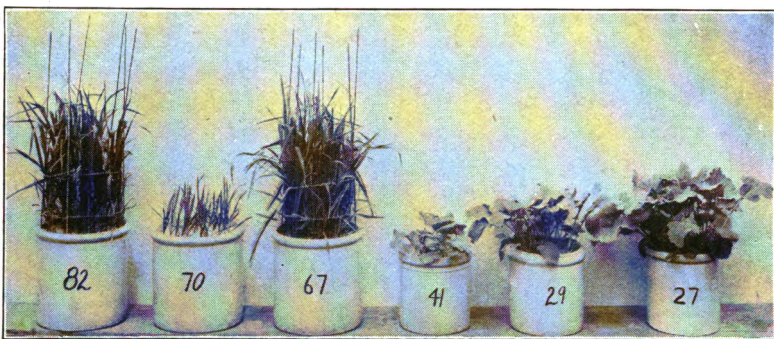


FIGURE 10. AVAILABILITY OF IRON PHOSPHATE TO OATS AND RAPE

Oats made nearly as good growth on ferric (iron) phosphate (82), as on acid phosphate (67), the growth being much better than on rock phosphate (70). With rape the results are strikingly the reverse, rock phosphate (29) producing much better growth than ferric phosphate (41).

phosphorus, though it is caused by the destruction of organic matter, comes, in large part, from iron and aluminum phosphates, which are, before the oxidation of the soil, protected from the action of the acid by being inclosed within particles of organic matter.

Mr. Truog of the Soils department has continued the study of the loss by leaching of phosphorus from heavily manured soils, and on the solvent action of organic matter on rock phosphate. He is also investigating the availability of the different forms of phosphate to various plants. In greenhouse tests oats made much better growth on freshly precipitated and dried ferric phosphate than on rock phosphate, while with rape the results were strikingly the reverse, as is shown in Figure 10.

## RELATION OF BACTERIA TO THE AVAILABILITY OF PHOSPHATES

During the past year Professor Tottingham of the Agricultural Chemistry, and Professor Hoffmann of the Agricultural Bacteriology department have continued their studies on the action of fermenting manures on phosphates added as reinforcing materials. They have found that fermentation over periods of from three to six months caused a marked decrease in the amount of water-soluble phosphorus in manure alone, or in mixtures of manure either with rock phosphate (floats), or with acid phosphate. In the case of the manure-floats mixture, less than half as much water-soluble phosphorus was found after fermentation as was originally present.

The addition of either chloroform or formaldehyde, which practically inhibited bacterial action, greatly reduced the decrease in water-soluble phosphorus in such mixtures of manure and floats, indicating that the loss occurring during fermentation was due to bacterial development. This view was supported by the fact that the growth of manure organisms upon media supplied with phosphorus in the form of the water extract from a fresh manure-rock-phosphate mixture caused a decrease of over 40% in the amount of water-soluble phosphorus, a loss comparable to that which occurred in the fermenting mixtures of manure. It was further found that about half the phosphorus in fresh, intact bacterial cells was apparently in the form of insoluble compounds (nucleins).

In conformity with the laboratory results, in greenhouse trials with barley, a 37% greater yield of grain was secured by the simultaneous application of fermented manure and soluble phosphate, than by the use of a mixture of manure and soluble phosphate which had previously undergone fermentation. On the other hand, in a second crop grown upon the same soil without any further fertilization, the fermented mixture proved superior, this reversal of results indicating that the phosphorus rendered insoluble by bacterial action was now available to the crop.

In similar trials in which a fermented mixture of manure and insoluble phosphate was compared with freshly mixed manure and phosphate, no difference in yield was obtained in the first crop, but in the second crop the fermented mixture produced the greater yield. This seems to indicate that the phosphorus

changed by fermentation, though less soluble in various chemical solvents, was more available to the second crop.

#### EFFECT OF LEVEL OF SULFUR SUPPLY ON PLANT GROWTH

As has been previously reported, Professor Hart and Mr. Peterson of the Agricultural Chemistry department found by improved methods of analysis that the sulfur content of cereals is nearly as high as their content of phosphorus, while other plants may contain much more sulfur than phosphorus. Their work also indicated that three times as much sulfur may be lost in drainage waters as is brought to the soil in rain. Analyses of cropped soils and adjacent virgin soils showed that the cropped soils contained about 40% less sulfur. These data therefore raise the question as to whether the natural soil supply of sulfur is adequate for permanent crop production.



FIGURE 11. SULFUR FERTILIZATION INCREASES GROWTH OF RAPE

Sulfur, as well as nitrogen, phosphorus, and potassium, may be a limiting factor in the growth of certain crops. Boxes from left to right: no fertilizer; "complete" fertilizer (N, P, K); third and fourth boxes, complete fertilizer, plus sulphate; fifth box, sulphate alone.

To determine the effect of sulfur fertilization various plants have been grown by Professor Tottingham of the Agricultural Chemistry department under greenhouse conditions with and without the addition to the soil of sulfur fertilizers. In the case of rape and radishes, both plants high in sulfur, sulfur fertilization has had a marked effect on the yield of dry matter. With rape grown under greenhouse conditions on soil from the Hill farm, supplying a sulfur fertilizer (gypsum, or land plaster) in addition to nitrogen, potassium, and phosphorus gave an increase of over 30% in the dry matter of the crop, as is shown in Fig. 11.

#### INFLUENCE OF GREEN MANURING UPON GERMINATION OF SEEDS

Last year a southern student at the College reported the failure of some ten acres of cotton to germinate, where it had been



sown immediately after plowing under green clover. On the other hand, on a similar field planted with the same seed, but which had not been green manured, normal germination occurred. Professor Hoffmann has accordingly begun a study of the effect of green manuring upon the germination of seeds subsequently sown. In pot tests in the greenhouse he has incorporated with the soil an amount of green clover corresponding to that applied under field conditions, and has then sown various seeds, in all cases sterilizing one series of pots, while another was allowed to remain in a normal condition.

It has been found that the decomposition of the clover somehow affects cotton seeds, but does not have any material effect



FIGURE 12. GREEN MANURING PREVENTS GERMINATION OF COTTON

When 1.5% of green clover was mixed with the soil (Jar 2) or 3.0% (Jar 4), and the whole sterilized, germination was as good as where no green manure was added (Jar 1). However, when 1.5% (Jar 3) or 3.0% (Jar 5) of green clover was added, and normal decomposition allowed to go on, germination was prevented.

on the germination of corn, wheat, and clover. Two experiments conducted with flax have, however, shown a similar detrimental effect to that produced on cotton. The results so far secured indicate that the decomposition of green manures results in a reduction of the oxygen supply and an increase in the carbon dioxide present in the soil atmosphere. It is thought that this change in gaseous content of the soil prevents the germination of the cotton and flax seed, which contain a high percentage of oil, and so require more oxygen for germination than such seeds as corn, clover, and wheat.

#### INCREASE IN NITROGEN FIXATION OF SOIL DUE TO APPLICATION OF CARBOHYDRATES

Professor Hoffmann has also studied the effect of various soil treatments on the bacterial activity of the soil at the Hill farm.

In the studies on this soil he finds that the application of sugar markedly increases the fixation of atmospheric nitrogen by the soil organisms which are able to fix nitrogen in the absence of any legume. This increased activity of these bacteria produced an actual increase of nearly 1,000 pounds of nitrogen per acre foot in three years. Similar results, though not quite so striking, were secured by the application of starch to the soil. It is interesting to note that when kainit and floats were applied together with either sugar or starch, the increase in the nitrogen fixing power was not so marked.

#### BACTERIAL AND CHEMICAL FACTORS IN THE RIPENING OF CHEDDAR CHEESE

The studies in cooperation with the Dairy Division on the bacterial and chemical factors concerned in the manufacture and ripening of cheddar cheese have been continued by the departments of Agricultural Bacteriology and Agricultural Chemistry.

As previous investigators had found the group of lactic acid bacteria the only one of constant occurrence in cheddar cheese, it has been quite generally believed that no other single group or at least single species of bacteria was absolutely essential to the ripening process. Professor Hastings and Miss Evans have found, however, that following the period of activity of the lactic bacteria, the group of high-acid-forming bacilli, or rod-shaped organisms, always make their appearance and gradually increase in numbers until they form 50 to 99 per cent of the total bacterial content. In 11 out of 13 cheese examined the coccus or round forms of bacteria have also been found to predominate at some time, varying from the 14th to the 161st day, making it probable that these forms are constantly present in large numbers in cheddar cheese.

Over 250 bacterial cultures, isolated from normal cheddar cheese, have been studied in detail and arranged in groups according to their power to ferment various carbohydrates. The results so far secured tend to show that the high-acid bacilli which ferment the more resistant test substances give a rank flavor to the cheese if they develop in very great numbers, but on the other hand, it requires lactic bacteria or cocci which do ferment some of these more resistant substances for the development of the best cheddar flavor.

In making cheddar cheese, as a guide in the amount of starter, to be added to the milk and also in subsequent operations, it is important to know as accurately as possible the rate at which acid will be developed during the process. Of course, this depends upon the number of lactic bacteria present in the milk. During the so-called "period of incubation" of the milk, while the bacterial content may be increased many thousand fold, the acidity remains stationary, so far as can be determined by titration methods. In these studies a considerable number of tests has shown that small differences in acidity are much more easily detected by the rennet test than by titration, and hence, the rennet test is a much more delicate means for the cheese maker to use in determining the condition of his milk.

It seemed very questionable from preliminary work whether the production of flavor in cheddar cheese was alone due to the decomposition of the casein, as such decomposition might be quite complete and yet no typical flavor appear. The chemical studies on the factors involved in the ripening process, carried on during the past six years under the direction of Professor Hart, have therefore been confined to studies of the non-nitrogenous constituents occurring in cheese. In the earlier work, reported in Research Bulletin 11, it was demonstrated for the first time that certain volatile compounds (esters) were progressively formed during the ripening process. Volatile fatty acids and alcohols were also found in varying amounts in normal cheese. These non-nitrogenous compounds, which were important in producing a characteristic cheese aroma, were not formed in chloroformed cheese or milk.

During the past two years, Mr. Flint, the chemist detailed by the Dairy Division, has studied the occurrence of these non-nitrogenous constituents in good and poor cheese, in an effort to determine the cause of the difference in flavor. No very definite relation was found to exist between the various kinds of these volatile compounds and the flavor of the cheese. Nevertheless, since there are differences in these constituents of the cheese which can have been produced only by bacterial activity, a study is being made of the by-products produced by pure cultures of the various groups of organisms normally present in cheese. It is hoped that it may be demonstrated that certain bacterial types are responsible for the production of definite compounds which cause flavor.



It has been found that representatives of the coccus group which are abundant in cheese at some time during the ripening process, produce large amounts of volatile acids. Most of these organisms also produced alcohol, but only two were found capable of forming esters. The origin of ammonia in normally ripening cheese has never been hitherto satisfactorily explained, but in these studies it has been found that representatives of both the coccus and the high-acid-producing groups are able to produce this substance.

In this work considerable time has of necessity been devoted to perfecting methods of determining the various compounds. Improvements have been made in the methods of separating and determining active and inactive lactic acid, and also in the Duclaux method for the estimation of volatile fatty acids.

#### CHEDDAR CHEESE FROM PASTEURIZED MILK

The presence of inferior cheddar cheese on the market, and the lack of uniformity which characterizes the product of the present average cheese factory, is due primarily to the variable quality of the milk supply from different farms. In an attempt to produce cheese of uniform quality from milk of variable acidity, which is often contaminated with undesirable bacteria, cheesemakers use methods which vary from day to day, carefully watching each vat at every stage of its manufacture and modifying the process to meet the conditions. Even then the quality and also the yield of cheese varies from day to day.

In an endeavor to devise a process for treating milk daily at the factory to bring it into practically a uniform condition for cheesemaking purposes, Professor Sammis of the Dairy department has developed a process whereby the milk is pasteurized, and then brought to uniform acidity by the addition of a small amount of hydrochloric acid, after which a pure culture starter is added to carry forward the cheddar process. This work has been carried on in cooperation with the Dairy Division of the United States Department of Agriculture.

This process has been carefully compared with the regular factory process for three years at the University creamery, the cheese being cured under different conditions and marketed in many sections of the country.

A greater yield of cheese has always been obtained from pasteurized milk than from raw milk, the average gain in yield of green cheese being 5.37% in 1911. The pasteurized milk cheese varied much less in quality, and averaged better by 3.7 points of total score than the raw milk cheese made from the same milk supply. The cheese sold readily for the ruling market prices, and often above. Even after storage under adverse conditions for one month at New Orleans at a temperature of 75° to 80°F, the pasteurized milk cheese averaged three to eight points better in total score than the raw milk cheese. Pasteurized milk cheese can be cured without injury at 70°F.

Before recommending this new process for general use, it will be given a thorough trial in cheese factories in various localities to test its applicability to different milk supplies. During the past season, the method has been tested in the Prairie factory at Spring Green, one portion of each day's milk being made into cheese by Mr. Bruhn, the cheesemaker detailed by the U. S. Dairy Division. The same gain in yield of pasteurized cheese has been obtained as at Madison, the gain being especially high on days when the raw milk curds were gassy. In so far as uniformity of flavor was concerned, the pasteurized milk cheese was superior to the raw milk cheese, but on many days the pasteurized cheese made at Spring Green contained a great many small holes, usually called "Swiss holes" or "fish eyes," a defect which had never been encountered with the milk supply received at Madison. Efforts are now being made to remedy this defect.

#### DAIRY MANUFACTURING TESTS AND APPLIANCES

During the past year Professor Benkendorf of the Dairy department has perfected a simple piece of apparatus which can be used successfully for measuring the overrun or "swell" in ice cream making, and has also designed and perfected a new way of using a burette for calibrating milk and cream test bottles.

#### MOTTLES IN BUTTER

Professors Sammis and Lee of the Dairy department have continued their work, begun a little over a year ago, on the cause of mottles in butter. By emulsifying dry butter fat, previous-

ly freed from casein by filtering through paper, with water by means of the homogenizer, and then salting the product, butter was produced which showed typical mottles when the salt was not evenly distributed throughout the mass. Mottling of butter may thus be produced entirely independent of the casein. A characteristic sample of this mottled casein-free butter is shown in Figure 13. Examination of such butter under the

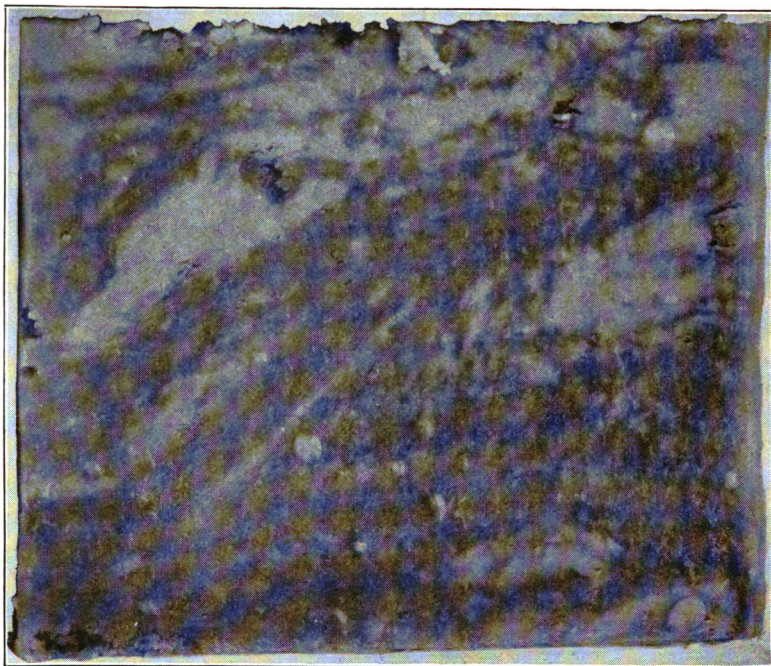


FIGURE 13. MOTTLED, CASEIN-FREE BUTTER

This marbled condition seriously deprecates the value of butter. Thorough working is important in preventing this fault.

microscope shows that in the portions which are lighter in color, the water is present in the form of innumerable minute droplets, thus rendering these layers opaque, while in the darker portions, the droplets of water are much larger but fewer in number, thus rendering the butter more translucent.

The results secured in these experiments throw a new light on the whole subject of "mottles" in butter and emphasize the importance of thorough working of the butter to prevent the production of this mottled appearance which seriously depreciates the value of the product.

### ICE CREAM MANUFACTURE

Hitherto little scientific study has been given to the manufacture of ice cream, the various methods and practices resting merely on an empirical basis. Mr. Baer of the Dairy department has begun a study of the value of different modes of procedure, conducting experiments with both the stationary and continuous type of freezers under conditions similar to those employed by commercial manufacturers. Only by such careful studies can this rapidly growing industry be placed upon a scientific foundation.

### PURIFICATION OF CREAMERY SEWAGE

The effluent from septic tanks used for the decomposition of creamery sewage when run onto filter beds usually has a very strong odor, which makes the use of such filter beds objectionable in an inhabited neighborhood. During the past two years Professor Farrington has successfully used a one per cent solution of common bleaching powder for deodorizing both the effluent from the septic tank at the University creamery, and the raw sewage in the drain at the Verona creamery station. The decomposition of the sewage is not checked by the use of this solution, but the offensive odor is greatly diminished.

The deodorizing solution is made by mixing one pound of bleaching powder with 100 pounds of water and allowing to settle. The clear liquid is then drawn off and sprinkled over the filter beds in about the proportion of one gallon to 150 cubic feet, or 1100 gallons, of sewage.

### EFFECT OF RATIONS FROM SINGLE PLANT SOURCES

To determine what would be the specific physiological action of rations restricted to single plant sources, upon cows subjected to the strain of reproduction, an experiment was undertaken five years ago by the departments of Agricultural Chemistry and Animal Husbandry. Young heifers were fed chemically balanced rations from the corn, the oat, and the wheat plant. As the animals reached physiological maturity and underwent the strain of reproduction, it became evident that the ration from the wheat was strikingly deficient, the wheat-fed mothers producing either dead or weak, undersized offspring. The results

of the earlier work on this problem have been reported in Research Bulletin 17 and in preceding Director's Reports.

During the past year attention has been concentrated on attempts to determine the cause of the disastrous effects of the wheat ration. In addition to the ration prepared exclusively from the wheat plant, rations were fed consisting either of wheat grain or of wheat straw, together with parts of other plants. For example, offspring have now been produced by cows on the following rations: (1) Wheat grain and corn



FIGURE 14. CALF OF COW FED WHEAT STRAW AND CORN GRAIN

Calf dead at birth.

stover; (2) corn grain and wheat straw; (3) corn grain with equal parts of wheat straw and alfalfa hay. Upon rations consisting of the wheat grain and corn stover, normal, healthy calves were produced. As soon as wheat straw formed the sole roughage, no matter what grain was used, invariably the urines became acid and weak undersized offspring resulted. However, upon the ration consisting of the corn grain with equal parts of wheat straw and alfalfa hay, normal calves were produced.

These results tend to indicate that the deficiency of the wheat plant is not due to toxicity of any part of the plant, or to an insufficiency of the proteins, but rather to the acid condition



imposed on the animal, caused by an insufficient supply of lime and other alkaline substances in the roughage. While this disastrous effect of the wheat straw was overcome by the addition of alfalfa hay, especially high in such alkaline substances, previous work indicates that it cannot be remedied by the addition of alkaline carbonates. Work is being continued along various lines to throw further light on these problems of great physiological importance.

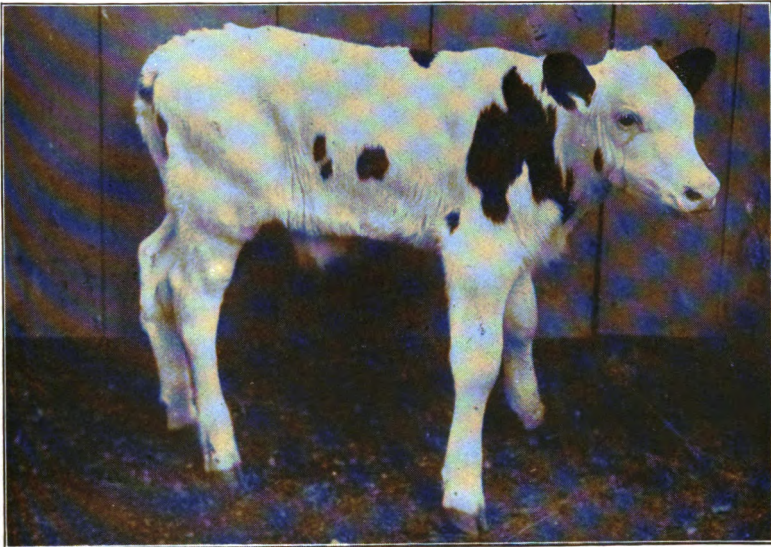


FIGURE 15. CALF OF COW FED CORN STOVER AND WHEAT GRAIN  
A vigorous, thrifty calf, normal in every way.

The same line of work was inaugurated with chickens last year by the Agricultural Chemistry and Poultry Husbandry departments. As no roughage could be employed in this case the rations were limited to (1) wheat grain and its products; (2) corn grain and its products; (3) oat grain and its products; and (4) barley and its products. The pullets which were limited to the wheat grain and its products made as rapid growth and reproduced chicks with apparently as great vitality as pullets fed rations from any of the other grains. There were differences in the number of eggs produced on the various rations, but individuality, as well as feed, may have been a factor in producing this result.

## MINERAL REQUIREMENTS OF ANIMALS

The Agricultural Chemistry and Animal Husbandry departments during the past year have continued their studies on the mineral requirements of farm animals, special attention having been given to questions concerning lime (or calcium) and phosphorus. The claim has often been made that feeding to pregnant animals a ration high in mineral matter, especially lime, will cause undue development of the skeleton of the fetus, with subsequent difficulty at birth. This problem has been thoroughly studied with swine by feeding brood sows rations high in lime. The results show that no such thing occurs, but that the size and lime content of the fetus skeleton is kept very constant when widely different amounts of lime are consumed in the food.

Further data on the lime requirements of farm animals have been collected on growing and mature swine, and on a mature goat, both dry and in milk, as it was apparent that the amount of lime demanded would turn on whether the animal was growing, producing milk, or merely maintaining itself. An interesting observation in this work was that the bulkiness of the ration determined to a considerable extent the amount of lime lost in the feces. In other words, a hard pressed animal machine, as a milch cow or goat, with a very large consumption of food and a correspondingly large fecal residue, will lose a large amount of lime from the body in the feces. This emphasizes the desirability of liberal supplies of lime in the rations for such animals.

It was formerly believed that animals could only build the important phosphorus containing organic compounds in the body tissues from similar organic compounds furnished in the food. Thus fish was considered a superior brain food, since it was high in organic phosphorus compounds. Professor McCollum has studied this problem with the hen, which in the egg produces large amounts of lecithins (phosphorus containing fats.) Eggs containing normal amounts of lecithins were produced on rations which contained no such compounds. This experiment, together with Professor McCollum's previous work in which he showed that rats could make appreciable growth on rations containing only inorganic phosphorus, show that the animal has much greater power of building the organic compounds found in its body than has generally been believed.

In studying certain fundamental problems in animal nutrition, Professor McCollum is conducting numerous experiments with growing rats, supplied with distilled water and fed various organic nutrients together with inorganic salt mixtures. He has not been able to produce normal growth with young rats on any natural grain, but has secured normal growth on skim milk powder, and on egg yolk. Although rats did not grow when fed the wheat kernel alone, or the wheat kernel plus wheat gluten, when mineral salts were added so as to make the total content of the mineral matter in the diet closely similar to that of milk powder, normal growth was produced for 70 days. Rats grew normally for 75 to 100 days on a ration consisting of pure casein and dextrine, if salt mixtures were supplied, which made the mineral content of the ration similar to that of either milk or egg yolk. However, when the same casein and dextrine mixture was fed with a salt mixture which gave the ration a content of mineral matter closely similar to that of the wheat grain, growth was suspended completely.

On either the last ration or on the ration consisting of wheat and wheat gluten, both of which contain a large amount of magnesium compared with the amount of calcium present, growth was induced in some degree both by the addition of calcium, or by the subtraction of magnesium.

These experiments show that not only must sufficient amounts of the necessary mineral elements be supplied, but also that there must be a proper balance between them, a fact hitherto not sufficiently recognized. Since the mineral content of egg yolk is highly acid and that of milk slightly alkaline, yet both lead to normal growth, it is evident that for this species an acid diet is not injurious. This may not be true for herbivora.

#### INFLUENCE OF SULFUR IN FEEDS UPON WOOL PRODUCTION

As practically all proteins contain considerable sulfur, in all probability farm animals in general obtain sufficient sulfur in their usual rations. The sheep, however, produces in its wool a product unusually rich in sulfur. This fact, together with the practice of good shepherds of supplying their flocks feeds especially rich in sulfur, suggested that a large intake of sulfur might be conducive to the production of an increased yield of wool or wool of better quality.



Accordingly during the past three years experiments have been carried on by the departments of Agricultural Chemistry and Animal Husbandry in which four lots of four sheep each of the wool type have been fed a ration as low in sulfur as could be made from the ordinary farm grains and hay; the same ration, plus sugar beets, a succulent feed low in sulfur; also the same ration, with rutabagas, a succulent feed high in sulfur; and the same basal ration with calcium sulfate, as a source of inorganic sulfur.

It has been found that neither the average gross weight of the fleeces, nor the proportion of pure wool fiber to the total weight was greater on the high-sulfur than on the low-sulfur rations. The percentage of sulfur in the pure fiber of the various lots was practically the same, as was the yolk in the fleeces.

It is evident from the results of these experiments that the normal dry rations of grain and hay contain ample sulfur for wool production, and that additional supplies seem to have no influence upon the proportion of pure fiber formed.

#### INFLUENCE ON GROWTH OF SWINE OF AMOUNT OF PROTEIN FED

Numerous practical feeding trials have led animal husbandry workers to hold that growing animals need, for the most economical production, a relatively narrow ration, i. e., one high in protein. During the past year Professor McCollum of the Agricultural Chemistry department has conducted metabolism experiments with pigs to determine the influence of the amount of protein in the ration, other factors remaining constant, on the tendency of young pigs to retain nitrogen for growth. All the animals received rations supplying the same amount of energy per pound of body weight, but the protein supply was fixed at 5, 10, 15, or 20 times the amount required for maintenance. When five times as much protein was fed as was required for maintenance, making a ration having a nutritive ratio of 1:11, only 10% of the nitrogen fed was retained. When twice as much protein was supplied in the food, 10 times the maintenance requirements being fed, or a ration with a nutritive ratio of 1:5.5, 23% of the nitrogen in the food was stored up in the tissues. When the amount of protein fed was increased to 15 to 20 times the amount required for maintenance, the same percentage was still stored. In other words, when the very narrow nutritive ratio of 1:2.7

was fed, supplying 20 times the protein required for maintenance, over twice as large a percentage of the protein fed was stored for growth as on the wide ration which supplied only 5 times the maintenance requirements.

#### VALUE FOR GROWTH OF NITROGEN IN ALFALFA HAY

For many years a problem of great dispute among scientists has been the question of whether animals can use for growth the so-called amid nitrogen, which is present to a considerable amount in such feeds as roots, silage, and hay, in the same manner as they use the nitrogen in the form of the more complex true proteins. As one-fourth of the total nitrogen of alfalfa hay is in the form of this amid nitrogen, it is a matter of considerable importance in the use of this feed as a source of protein, to learn whether all of the nitrogen is of full value, or whether the amid nitrogen must be considered worthless for growth and other vital purposes.

To throw more light on this question, experiments have been carried on during the past two years by the departments of Agricultural Chemistry and Animal Husbandry. In these trials young, growing heifers were fed rations in which alfalfa hay was the sole source of nitrogen, while others were fed rations from the corn plant, in which practically all of the nitrogen was in the form of crude protein. Long continued experiments have now been conducted with four different animals in which accurate records have been kept of the intake and outgo of nitrogen. Since these growing heifers uniformly stored as high a percentage of the total nitrogen of alfalfa hay as they did of the corn plant, we must conclude that the amid nitrogen was not worthless for growth, but that with these animals the entire nitrogen of alfalfa hay was of full value.

#### LESSONS FROM THE WISCONSIN DAIRY COW COMPETITION

As a part of the general campaign for the advancement of Wisconsin dairying, the Wisconsin Dairy Cow Competition was conducted by Professor Woll for a two-year period ending November, 1911. Of the 506 entries made in this competition, complete yearly records of production and feed consumption were obtained from 395 animals. The immediate value of the contest to the breeders who competed, and to the live stock in-

terests of the state in general, has already been very great, both through the publicity received and the stimulus to individual breeders and the farmers in their respective localities. This value, important though it is, is insignificant compared with the influence which would be exerted if all Wisconsin farmers carefully studied the results and lessons secured in this work.\*

A most important fact brought out by the extensive data is that the large cows within each breed were, as a general rule, more economical producers than the small ones. Though the large animals were heavier eaters, their production was enough

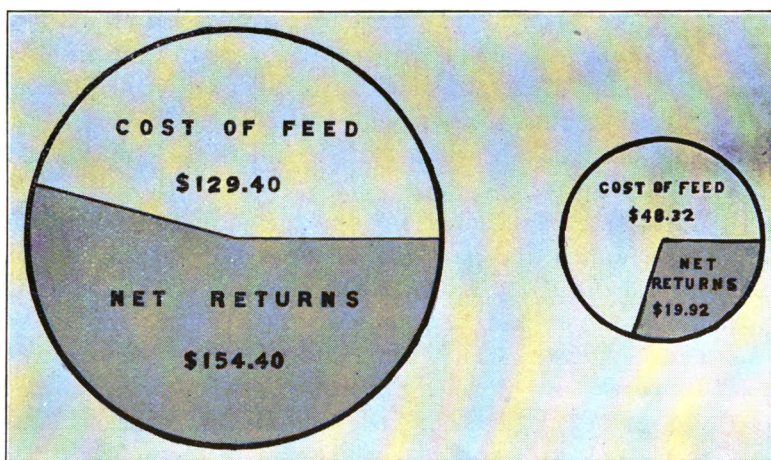


FIGURE 16. NET RETURNS FROM BEST AND POOREST COWS

The best cow consumed about two and three-fourths times as much feed as the poorest cow, but on account of her much greater production, her actual net returns were nearly eight times as great.

greater to make their net returns larger than in the case of the small animals.

The cow that ranked first in actual production of butter fat, when compared with the lowest producer in the Competition, consumed feed costing 168% more, but in return produced 882 pounds of butter fat, or over four times as much as the poorest one. In actual net returns the best animal was nearly eight times as profitable as the poorest cow. Who is there that would consciously feed, milk, house, and care for eight animals, in-

\*The general results are published in Bulletin 226 while the detailed data and the more critical study are given in Research Bulletin 26 of this Station.

stead of one, if he knew just what was taking place in his herd? This example is by no means extreme, since perhaps not more than one-third of the cows on Wisconsin farms yield better results than were actually obtained from this lowest producer in the competition, which gave nearly 4400 pounds of milk, and 218 pounds of fat.

A study of the rations consumed shows that the large producers ate from 18 to 26 per cent more total feed, and from 38 to 61 per cent more concentrates than the low producers. The feed of the former class was thus made up of a larger proportion of concentrates and of smaller proportions of pasture grass and hay, or other dry roughage, than that of the low producers. The results obtained in the competition illustrate in a striking manner the fact that a large dairy production cannot be secured except by furnishing a large supply of feed. The increase of 23% in the total amount of feed eaten by the 25 best cows in the competition, compared with the 25 poorest cows, was accompanied by an average production 78% greater than the poorer cows.

#### CORN SILAGE FOR BEEF CATTLE

Mr. Tormey of the Animal Husbandry department has conducted feeding trials for the past three years to determine the value of corn silage for fattening steers, with results which agree in showing that it is a most economical feed for this purpose. In the trial of last winter three lots of five 2-year-old steers each, were fed the same concentrate allowance (averaging 12.55 pounds of corn meal, 2.05 pounds bran, and .25 pound cotton seed meal), with roughage allowance as follows: Lot I, corn silage 32.83; Lot II, corn silage 7.32, alfalfa hay 8.83 pounds; Lot III, alfalfa hay 11.72 pounds. It should be stated that the steers were used for class room work, and hence subjected to more exercise and worry than usual, which doubtless had some effect upon the cost of putting on the gains.

During the feeding period of 104 days the steers fed silage as the sole roughage made the most economical and the largest gains, the average daily gain per steer being 2.73 pounds. In rapidity and economy of gains, Lot II, fed silage and hay as roughage, were second, producing nearly as large gains as the silage fed lot. In this short feeding period the steers finished

fully as well on corn silage alone as on alfalfa. After the steers got on feed no digestive troubles were experienced through the use of silage. Toward the end of the feeding period, however, the silage fed cattle had to be watched more closely than the steers fed either hay or hay and silage, and the allowance of corn silage cut down, owing to a tendency to get off feed. On the other hand, the steers in the silage-alfalfa lot were always greedy for their feed and consumed a large amount of roughage. The results show that it is profitable to feed silage as the sole roughage to fattening steers under the present average market conditions. This is especially true when the price of hay is high.

#### SILAGE FROM SUGAR BEET TOPS AND SHOCKED CORN

As about 250,000 tons of sugar beets are now raised annually in Wisconsin, the best utilization of the beet tops is of considerable importance. Last fall Professor Humphrey of the Animal Husbandry department ensiled this material with shocked corn. Though the silage had a slightly stronger odor than ordinary corn silage, it was not offensive. The cows seem to relish it, and did as well as on the regular corn silage. Chemical analysis showed that this silage had practically the same composition as clear corn silage.

After the beets were removed from the field, the tops, which had been left in small piles, were run through an ensilage cutter with an equal amount of corn fodder taken from the shock. By throwing the beet tops onto a layer of corn and cutting them both together, no difficulty was experienced. Enough water was added to the cut material to give it the proper moisture content, and make it pack well when two men tramped it during the time of filling. The production of carbon dioxide gas from the respiration of the fresh beet leaves is sufficient when the cut material is tightly packed to keep the whole from developing mold and other undesirable fermentations.

#### COST OF PRODUCING BUTTER FAT

The cost accounting work conducted by the Department of Agricultural Economics under the direction of Professor Taylor, in cooperation with the Office of Farm Management of the United States Department of Agriculture, represented by Mr. Juve, has been continued the past year to obtain further figures

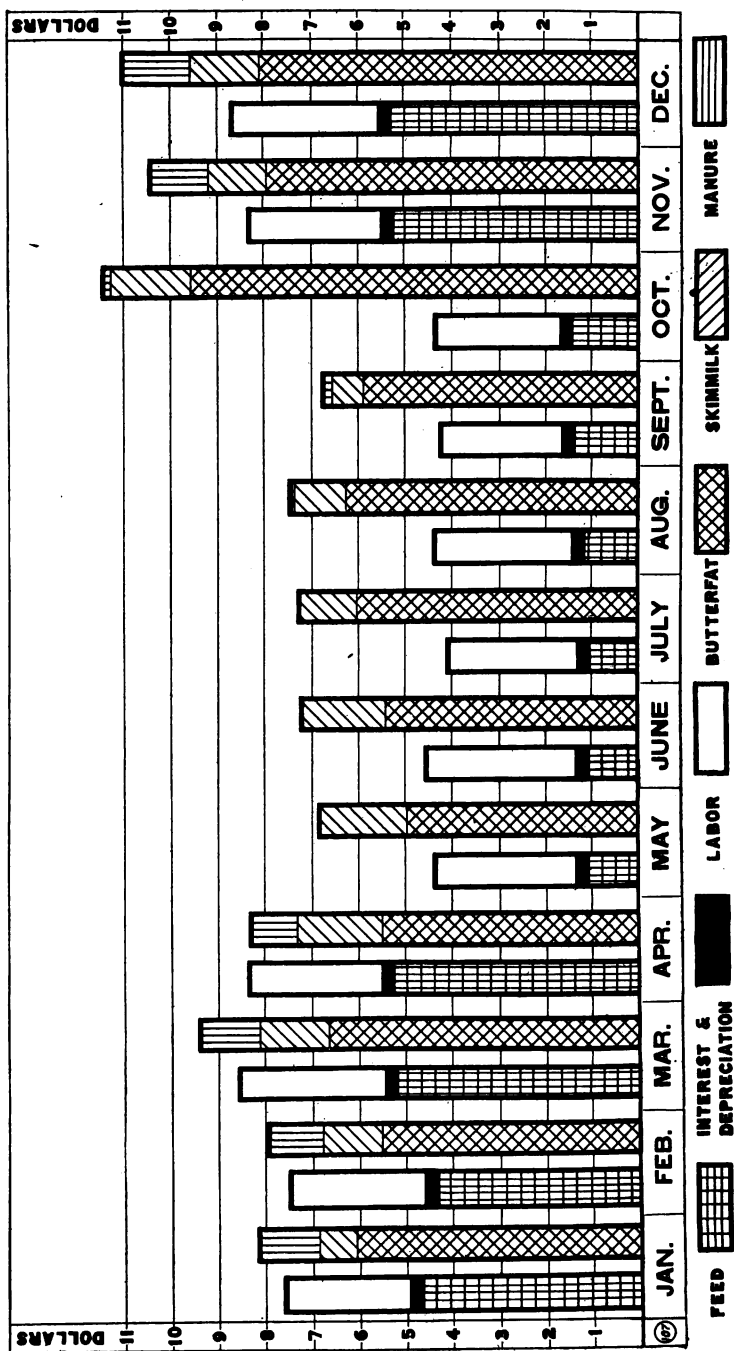


FIGURE 17. COST ACCOUNTING STUDY ON WISCONSIN DAIRY FARM  
 was not great enough to offset the increased cost of feed.  
 In this particular herd, the price of butter fat in winter

on the cost of producing farm products. Considerable attention has been devoted to the cost of producing butter fat, accurate daily records being kept on representative Wisconsin dairies. In Figure 17 are given the average monthly costs and returns per cow on one of these farms. In obtaining the records the herd is charged with the market value of feeds, with interest and depreciation of both herd and dairy equipment, and with the labor expended. The herd is credited with the value of the butter fat at average monthly Elgin prices, with the skim milk at 20 cents per 100 lbs., and with the actual value of the manure produced.

The most striking feature of the chart is, perhaps, the difference between the costs and returns for the winter and the summer months. Both the feed cost and the price of butter fat go up in the winter months, but on this specific farm the rise in price of butter fat was not great enough to offset the increased cost of feed. Therefore, figuring labor at the same price per hour for winter and summer, greater profit was secured in the winter months.

It should be noted, however, that the dairy herd did furnish fairly-profitable employment for the farm labor in the winter, when doubtless there was opportunity for no other employment which was even so profitable.

The records gathered have shown clearly that the factors of cost vary widely on different farms, and hence this chart can not be used for drawing general conclusions. Such data are, however, most valuable on the individual farm where they are obtained, in determining what to produce and when to produce it.

#### HISTORY AND GEOGRAPHY OF AMERICAN AGRICULTURE

Professor Taylor of the Agricultural Economics department has continued his studies on the history and geography of American agriculture, collecting data from all sources to tell the story of the expansion of various lines of agriculture. A set of about 300 maps has been constructed showing the distribution of every line of production in 1909 and of all the important kinds of farm property in 1910.

Figure 18, showing the distribution of dairy cows in the United States in 1910, is of especial interest because it well shows the rank of Wisconsin as a dairy state. At this time Wisconsin had over 1,470,000 dairy cows, being then outranked

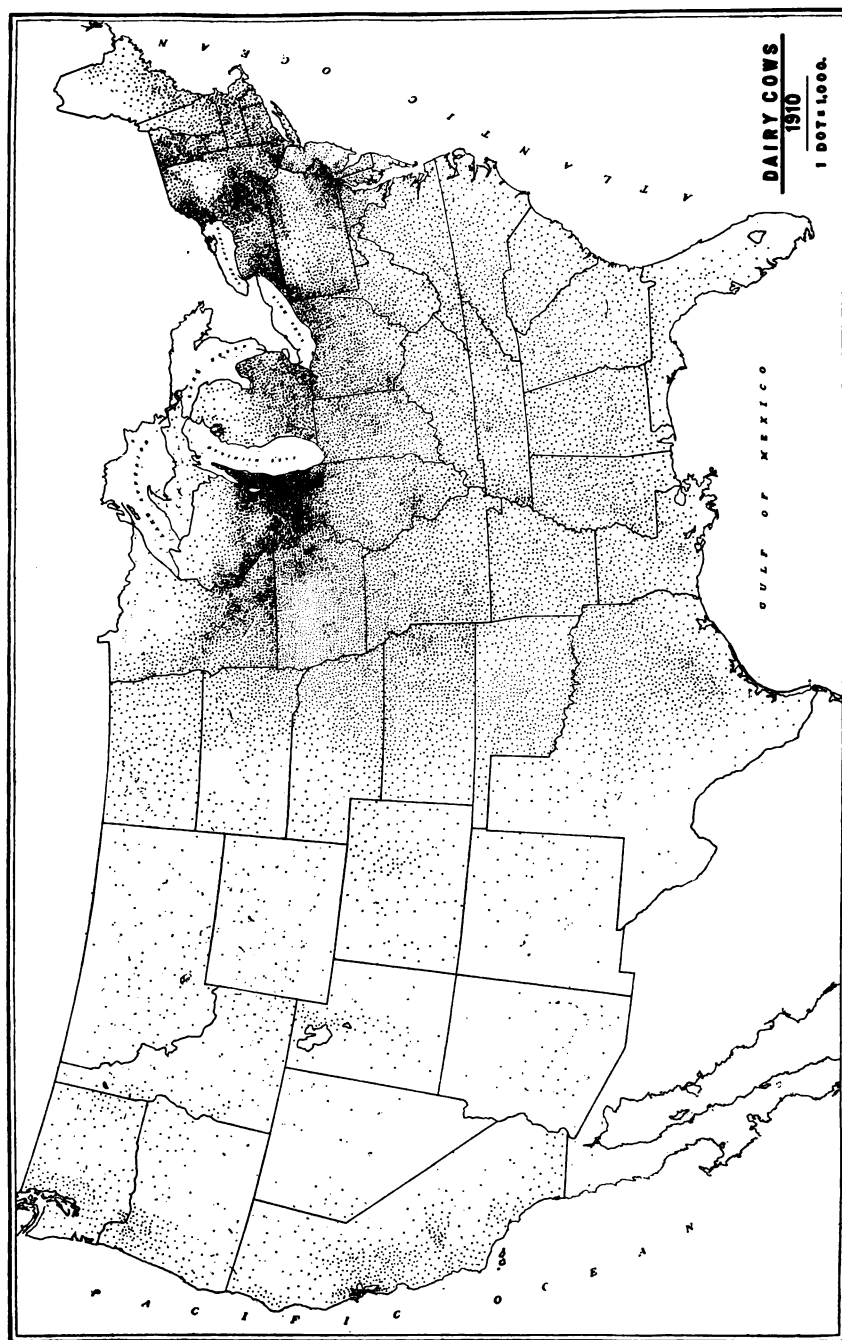


FIGURE 18. DISTRIBUTION OF DAIRY COWS IN UNITED STATES (CENSUS OF 1910)

Wisconsin now leads all other states in number of dairy cattle. We have more than one cow for every two persons in the state.



only by New York, which had 38,000 more head. During the preceding 10 years the dairy cattle of Wisconsin had increased 47%, while New York had remained practically stationary, showing a gain of less than one per cent. On the assumption that this rate of increase has continued, it is evident that Wisconsin now ranks first in this respect among the states of the Union.

#### EFFICIENT DISTRIBUTION OF FARM CAPITAL

Professor Otis of the Agricultural Economics department has continued his farm management survey, gathering data on Wisconsin dairy farms concerning the amount and distribution of capital, the amount and sources of farm income, and the expenses. Data obtained from 80 farms, on which the total invest-

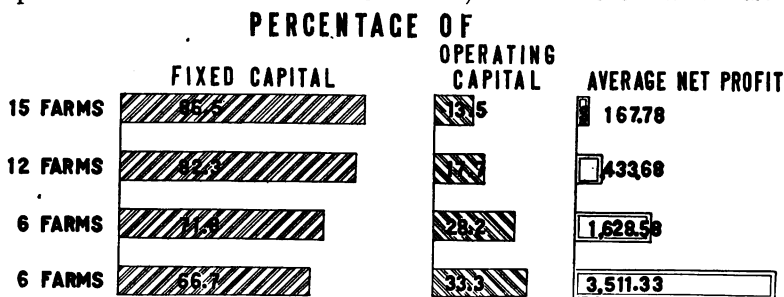


FIGURE 19. RELATION BETWEEN DISTRIBUTION OF FARM CAPITAL AND NET PROFITS

On the 39 Wisconsin farms here represented, the net profit was greatest where the investment in operating capital was from 20 to 33 per cent of the total capital.

ment of capital ranged from about \$10,000 to over \$60,000, indicates that there is a close relation between the percentage of operating capital and the net profits or management income from the farm. On these farms the best success was obtained where the operating capital, consisting of investments in live stock, machinery, tools, cash balance, etc., ranged as high as twenty to thirty per cent of the total capital, as is shown in Figure 19. In other words, to insure maximum profits, the operating capital must be sufficient to equip the farm thoroughly and properly.

#### MARKETING OF WISCONSIN CHEESE

While in 1889 Wisconsin produced only 21 per cent of the total amount of cheese made in the United States, in 1909 she

ranked first in the Union with about 149,000,000 pounds, or 46.6 per cent of the total production of the country. Owing to the importance of the cheese industry, at the suggestion of the State Board of Public Affairs, a study of the marketing of Wisconsin cheese was begun by the Agricultural Economics department under the direction of Professor Taylor. All steps of the marketing process will be taken up, from the time the cheese leaves the factory until it reaches the consumer. Especial attention has been given to the history of the dairy boards, and a statistical study has been made which shows the primary destination of the cheese shipped from Wisconsin. An accurate picture of the business as it actually exists is the necessary basis of further suggestions for improving the methods of marketing.

#### A SOCIAL SURVEY OF WALWORTH COUNTY

A social survey of a rural community will raise, and in some degree answer, many important questions. For example, how necessary to the farming population is the adjoining village? Why do some village enterprises and institutions serve the farm home more completely than others? Are there any important interests common to both village and farm which suffer for lack of an adequate mutual understanding on the part of villager and farmer?

To answer such questions as these, Mr. C. J. Galpin of the Agricultural Economics department is engaged in tracing what we may call the paths from the various social institutions of each of the twelve major villages in Walworth county to the farm homes surrounding each village. The paths from the village bank to the homes using that bank tell who belong to that banking community and to what extent the bank depends for business upon the intelligent development of the farms. Similarly the paths from the village high school, store, creamery, library, newspaper office, and church, back to the homes on the land, tell stories of the social conditions of the community.

The survey will show the extent of each rural community centering in a village; will show the comparative influence out among farm homes, of the various village institutions in the same village; and also will show the comparative influence out among farm homes, of the same kind of institutions in the different villages.

## AGRICULTURAL EXTENSION SERVICE

The last year has witnessed a material expansion of the extension activities, particularly with reference to the field work. As communities come to recognize the value of this service, increasing demands are made on the College. At the present time a great many requests for aid from individuals, as well as communities, have to be refused on account of lack of men to carry on the work.

The extension service is divided into—

- (1) The departmental activities which are mainly demonstrational in character and are carried on so far as possible under field conditions;
- (2) The county agricultural representative system, in which representatives of the College are located in the several counties to carry on field extension work;
- (3) The collective work, including the simultaneous services of several departments, that is given through the medium of extension courses and schools held mainly in the winter.

### I. DEPARTMENTAL ACTIVITIES

#### DIAGNOSIS OF CONTAGIOUS ABORTION

The past year the Veterinary Science department has co-operated with farmers wishing to eradicate contagious abortion from their herds and has used the new complement fixation test as a means of diagnosing the disease. This malady is of greater significance to dairymen than bovine tuberculosis, as it is fully as insidious, and heretofore could not readily be differentiated from non-infectious troubles. By means of this blood test, the fact can now be accurately determined whether a cow harbors the contagious abortion germ even before abortion occurs. Doctors Hadley and Beach have tested over 500 animals representing a number of different herds, thus aiding the owners in separating the diseased from the yet uninfected stock. In these herds 35% of the animals tested gave evidence of infection, but of course the majority of these tests were made on suspected cattle. It would seem that this test will be extensively used by intending purchasers to determine the actual condition of animals purchased, before they are introduced

into a new herd, just as prudent purchasers now insist on a tuberculin test.

Community breeders' associations could well utilize this test in their herds and thus be able to guarantee stock sold as free from this dread malady.

#### DISTRIBUTION OF HOG CHOLERA ANTISERUM

Owing to the widespread prevalence of hog cholera in the state the last year, the Veterinary Department has undertaken the manufacture and distribution of antiserum to be used as a preventive treatment for this disease. This serum is obtained from the blood of hyper-immunized hogs, that is, immune hogs which have been rendered still more immune to the disease by injections with blood from hogs very sick with cholera. The injection of the serum alone will confer immunity to the disease for three weeks to three months. A more permanent immunity is secured by the double method of vaccination which involves a simultaneous injection of the protective serum and a graduated dose of virulent hog cholera blood which is drawn from a diseased hog.

The manufacture of serum was begun about May 1, and already 135,000 cubic centimeters have been made and distributed at cost (about 30-50 cents per animal) through the veterinarians of the state. The continuance of the outbreak through this summer has kept up such a demand that it has been wholly impossible to make the serum fast enough. The main value of the serum is to prevent the disease. Where animals are already sick with cholera, it is not advisable to treat them.

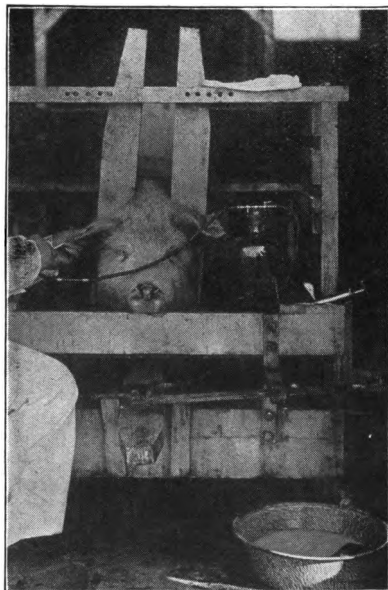


FIGURE 20. PRODUCTION OF HOG CHOLERA ANTISERUM

The losses from hog cholera constitute a leading factor in the high price of pork. The millions lost annually could be prevented by the use of this antiserum.

To encourage its use, Doctors Hadley and Beach have conducted a number of public vaccination demonstrations where the farmers have been invited to witness the application of the protective serum. It has been customary in a number of these cases to have the farmers appoint a committee of their own to report on the condition of the herd some time after the vaccination process.

#### DISTRIBUTION OF TUBERCULIN

Due to the marked public interest which has been aroused in this state through the medium of tuberculosis post-mortem demonstrations and the general propaganda which has been carried on as to the economic aspects of bovine tuberculosis, the demand for tuberculin for diagnostic purposes reached such proportions two years ago that it was impossible to secure sufficient material from the U. S. Department of Agriculture to carry on this work. The Board of Regents in January, 1911, therefore authorized the department of Agricultural Bacteriology to begin the manufacture and dissemination of tuberculin in response to this widespread demand.

Over 65,000 doses have been made and distributed to the farmers of the state through the medium of the State Live Stock Sanitary Board.

A comparison between the number of cattle tested in Wisconsin and in other states that are actively engaged in fighting bovine tuberculosis shows that from 1905 to 1911 in Pennsylvania 52,600 head of cattle (not including importations) were tested; in Minnesota, 135,021 for the period from 1906-1912; in Wisconsin, during the same period (1906-12) 353,611 cattle were tuberculin tested. The total number of cattle in Pennsylvania and Minnesota is a third greater than in Wisconsin, yet in our state in six years, 162,000 more cattle have been tested than in Minnesota and Pennsylvania for an equal period of time.

The cattle examined in Wisconsin have been tested under the auspices of the Live Stock Sanitary Board, the reacting animals being disposed of under federal inspection.

The law with reference to compensation for affected animals was modified in the last legislature, and after June 1, 1913, no compensation will be paid. It is expected that this action will induce many to test their herds this winter, so as to be able to

take advantage of the present policy of the state which aids the owner in eradicating this severe cattle scourge from his herds.

### BANKERS' PURE BRED SEED CONTESTS

Last year we announced the establishment of a cooperative plan with the Wisconsin Bankers' Association, in which the general Association was instrumental in arranging, through the medium of the local banks, for the distribution of pure bred seed to the farmers in their respective localities. The

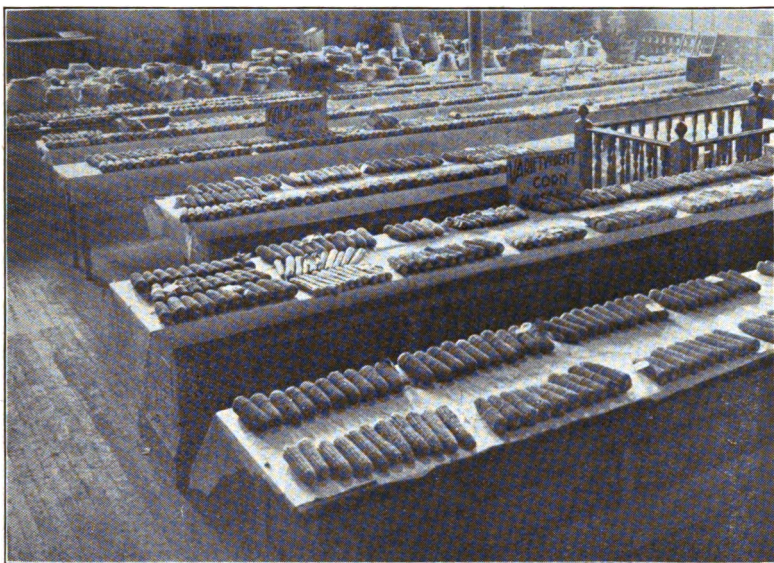


FIGURE 21. EXHIBIT AT EAU CLAIRE BANKERS' CONTEST

Over 3,100 exhibits made at 9 meetings attest the interest which has been shown by the farmers in this work.

local banks also arranged for competitive exhibits to be held in the fall. Before undertaking the enterprise the Bankers' Association requires the united effort of all banks in any town. The local organization furnishes the hall and defrays all incidental expenses, besides securing locally the prizes offered for the exhibits, while the College furnishes the necessary pure bred seed for dissemination, and speakers for the meeting.

During the winter of 1911-12, Professors Moore and Hatch have conducted nine such contests, holding very successful meetings in eight different counties. Over 3,100 exhibits were

put in competition, and the attendance aggregated nearly 6,500 persons. Aside from proving one of the most successful means of disseminating improved seed and accurate information relative to its culture, these meetings have been instrumental in coordinating the mutual interests of the town and the country. The stimulus which this work has imparted to the community is shown by the fact that a strong demand has sprung up from these localities for extension schools.

#### YOUNG PEOPLES' GRAIN GROWING CONTESTS

The work with the school children has been continued by the Agronomy department, cooperating with the county school superintendents and county fair secretaries. Forty-one contests have been held in 36 counties of the state. Considerable difficulty this year was experienced in getting suitable seed corn for distribution, owing to the poor seed obtained in the fall of 1911. Seed was distributed to approximately 18,000 children with specific directions as to care and culture of the crop.

As the result of the contests held in the fall and early winter, scholarship prizes were distributed which permitted the winner to attend the Boys' One Week's course at Madison during the Farmers' Course. Last year 52 boys were in attendance on this school, and as usual the keenest interest was manifested by these scholars in the work of the school, and incidentally in the work of the University.

In a considerable number of cases, young boys who have taken part in this work in former years are now going into the pure bred seed business. The case of Pierce Martiny of Baraboo typifies the results that flow from this line of extension activity. This youngster had become interested in these corn contests and had won recognition at the local fair. Through his success, the father was induced to use the improved variety of corn with which the son had won. Last year, the boy came to the Boys' Course, and in partnership with his father had enough seed corn so that he sold over \$500 worth at the time of the Farmers' Course. This fall he and his father have over a thousand bushels for sale. Already a seedman has contracted and paid the boy \$2.00 a bushel at wholesale for 600 bushels. Verily, "a little child shall lead them."



## DISSEMINATION OF PURE BRED SEEDS

For a number of years, the Agronomy department has co-operated most closely with the Wisconsin Experiment Association in the matter of disseminating improved strains of seed grains that have been perfected at the Station through long years of selection. During the last few years the pedigree strains that have been developed from most carefully selected



FIGURE 22. WORLD'S CHAMPION BARLEY

For three successive years the Wisconsin pedigree barleys have won the highest awards in open-to-world classes.

single plants have been brought to a state of higher perfection than obtained with the selected strains previously distributed.

A year ago pedigree barley and fall rye were sent out, and 231 reports on barley received this year show an average yield of 30.7 bushels per acre, or 3.6 bushels more than the average yield of all competing varieties. Forty-six reports with rye show an average yield of 24.5 bushels per acre for pedigree types in comparison with slightly less than 20 bushels for all other varieties. Since last year the Wisconsin Pedigree Barleys have been exhibited by Wisconsin growers at the International Barley Show



at Chicago in competition with the best barleys produced in other states and foreign countries. The World's Championship in the 6-rowed class, as well as the second prize, went to Wisconsin growers who are successfully propagating these pedigree types that Professor Moore has developed.

#### ALFALFA PROPAGANDA

The department of Agronomy has cooperated with the Wisconsin Experiment Association in organizing a special order of that Association to develop interest in growing and disseminating alfalfa, that queen of forage plants. This alfalfa order was organized only one year ago with 21 charter members, and now has a membership of over 500. Realizing the difficulty in getting seed of good germination and free from noxious weeds, Mr. Graber of the Agronomy department, who has acted as Secretary of this order, took up the matter of co-operative buying of seed for the members of the order. In this way not only was the very highest grade of seed selected, after passing rigid tests, but the placing of a single order for 19 tons of seed, amounting to about \$7,000, enabled members of the order to secure their seed at a saving of from 15 to 30%.

Trial tests are made by members of the order on thick and thin seeding, the use of lime to correct acidity, and the inoculation of soils with bacterial cultures or soil from alfalfa fields. These widespread tests which are now in progress will not only give most valuable data as to best methods of practice, but are conducive to the most rapid spread of alfalfa culture.

Already the culture of this plant has been greatly extended throughout the state, especially in the southern and eastern portions. Professor Moore estimates that now over 30,000 acres are grown, while 20 years ago it was practically unknown.

#### FARM CROP DEMONSTRATIONS

Field demonstrations have been conducted this past year on fifteen county and three state institutional farms by Professor Norgord of the Agronomy department. This demonstrational work which was originated in cooperation with the State Board of Control, has now grown to be one of the most important of our extension activities. Fifteen meetings have been held on as

many county farms during the late summer and fall at which there was an attendance of 7,350 people.

These meetings partake of the nature of a community enterprise, and a lawn picnic is generally held. To make the meetings of more direct interest to the women who attend, speakers have been secured at six of these meetings to present demonstrations of some phase of domestic science work. The well equipped kitchens with which these institutions are provided afford excellent facilities for such work.

The interest manifested by the farmers in this county farm work continues to grow year by year, and the most cordial cooperation has been shown by the farm superintendents. About a dozen lines of field trials are in progress on these farms.



FIGURE 23. SOIL INOCULATION INCREASES ALFALFA YIELD

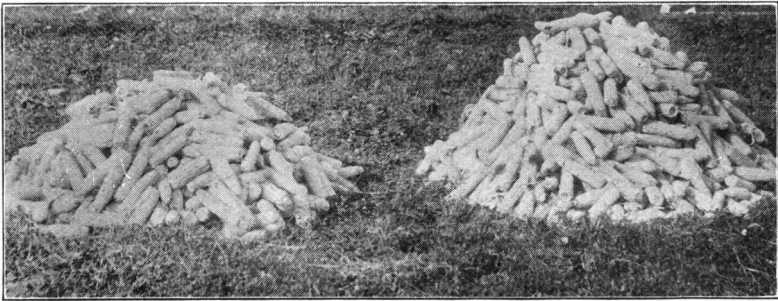
Actual yield (third cutting) on inoculated plot (right), and uninoculated plot (left), on the Viroqua county farm.

Owing to poor conditions which obtained in the fall of 1911, due to excessive rains, the seed corn situation in the state was quite critical. The time was therefore especially opportune to drive home the lesson of proper curing of seed. Comparative tests of farmers' seed were made in fourteen counties by means of germination tests and also ear-to-the-row plantings on the county farm, which showed the marked improvement to be observed where the seed was fire-dried or thoroughly cured.

On these farms pure bred seeds that have been originated at the Experiment Station are exclusively grown. Sixty-five thousand bushels were thus produced this year, and the excess seed has been sold chiefly to farmers in the neighborhood. Special emphasis has been placed the past year on the matter of

harvesting and storing grain so as to avoid the crop being wet by rains, thus preventing in considerable measure deterioration from molds and other fungus diseases.

Work on alfalfa has been continued as in previous years, liming and inoculation tests having been carried on. Figure 23 shows the third cutting of alfalfa on the Viroqua county farm as grown by Superintendent Butters. At the Tomah Indian School, Superintendent Compton grew 2,400 pounds per plot on an uninoculated field while the inoculated area yielded 6,035 pounds. Nearly 300 acres of alfalfa were grown this year on these county farms with an average yield of 4.2 tons per acre. Variety tests with Grimm's alfalfa compared



**FIGURE 24. MUCH PLANT FOOD IS LOST IN LEACHED MANURE**

Manure exposed to the weather over winter produced only two-thirds as much corn as fresh manure.

with Montana seed have not indicated sufficient superiority of the Grimm variety to warrant paying the higher price for seed.

The manure conservation work heretofore carried on by the Agricultural Chemistry department has been handled this year on these farms by Professor Norgord in consultation with Professor Hart. Striking lessons on losses due to leaching were shown in several places, an example of which is illustrated in Figure 24.

This year seven plowing matches have been held at which much interest has been developed.

#### WEED ERADICATION

The continued spread of noxious weeds is going on throughout certain portions of the state. During the last year, Profes-

sor Stone of the Agronomy department has, in cooperation with interested parties, carried on weed surveys in several counties to determine what species are most prevalent, the areas and regions infested, and the cost of weed control.

Experience has shown that Canada thistles may be controlled by the use of alfalfa, where soil and drainage conditions favor the growth of this forage plant. Professor Norgord has also been successful in eradicating quack grass and Canada thistles by the use of hemp where the seed bed is properly prepared by thorough preliminary cultivation. The very heavy growth of this plant on a fertile soil so completely shades the ground that these weeds are unable to gain headway.

A number of addresses have been given during the year on weeds and their eradication. Interest in this matter is rapidly developing, especially through the medium of the public schools. During the year, 1,033 samples of weeds have been identified and reported on by Professor Stone, in comparison with 425 specimens for the preceding year.

Something should be done to improve the legislation relative to weed eradication. As the matter now stands, the control over the spread of these pests is merely nominal. No really effective campaign can be carried on until this matter is put on the basis of state control, rather than leaving it to the initiative of the several counties.

#### THE DRAINAGE SERVICE

On much of the 7,000,000 acres in Wisconsin which need drainage, the excess of water is evident to the casual observer. But many experienced farmers do not seem to realize that a yellow spot surrounded by the rich green of a field of growing grain is frequently due to no other cause than poor drainage. During the past year, in the campaign of the Soils department for the reclamation of the land needing drainage, Professor E. R. Jones has aided, by making preliminary surveys and plans, in the organization of eleven drainage districts, including a total area of about 11,000 acres. In addition to these, the organization of seven districts which had been begun in previous years was completed. Thirty-three individual projects have also been laid out on different farms.

In carrying on this drainage work, it is the policy, before assistance is given in the organization of a district, to require

applicants to present requests signed by five or more land owners. During the organization, meetings of the land owners are held, at which various subjects concerning drainage are discussed. Assistance is given in individual farm projects only when the drainage problem on a particular farm is typical of those of a community. An effort is also made to have present at various stages of the work a number of neighbors whose land likewise requires drainage, so that as many as possible will be benefited.

As an instance of the benefits of tile drainage, followed by proper fertilization, the results on a peaty marsh near Stoughton may be cited. Ever since this marsh of 33 acres was tiled in 1908, at a cost of a little over \$20 per acre, the drainage has been almost perfect. The crop of buckwheat raised the first year after drainage and the crops of corn and sugar beets raised the next two seasons were more than satisfactory where fertilization and cultivation were right. In 1911 corn was planted on 30 acres which had practically all been fertilized with stable manure. Additional manure was applied when the corn was a few inches high on any spots where it appeared yellow, and the plants thus kept in rapidly growing condition. At harvest the field produced a yield of 92 bushels of shelled corn per acre.

The first two crops raised on the marsh so convinced the owner of the profit which might be derived from tiling such marsh that he purchased and tiled a nearby forty-acre tract. Due to lack of time the entire area was not fertilized in 1911 when the first crop was raised. The effect of proper fertilization was most striking, the fertilized portions yielding 100 bushels of corn per acre, while some of the unfertilized parts yielded only 25 bushels.

#### COOPERATIVE FERTILITY DEMONSTRATIONS

The Soils department has carried on the past year in cooperation with farmers in various parts of the state, 65 fertilizer demonstrations, 13 demonstrations of the benefits from correcting soil acidity by the use of lime, and 4 demonstrations on the methods of tillage. In the work in the southern part of the state on clay and marsh soils, in charge of Mr. Weir, especial attention has been given to the application of phosphorus fertilizers

and lime to acid clays to benefit the growth of clover and alfalfa, and to the use of potassium fertilizers on marsh soils. In the plots on the sandy soils, in charge of Mr. Ullsperger, the methods of building up these soils have been demonstrated, such as increasing the nitrogen content by the growth of legumes, supplying phosphorus and potassium through suitable fertilization, increasing the water holding capacity of the soil, and properly conserving moisture.

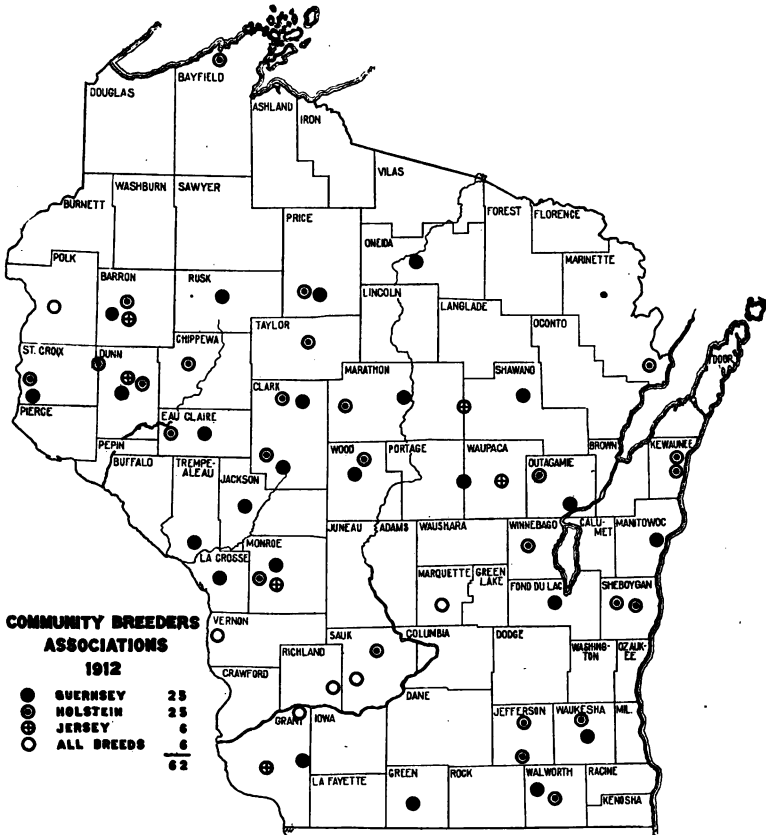


FIGURE 25. COMMUNITY DAIRY CATTLE BREEDERS' ASSOCIATIONS  
Cooperation in a community along a single line of effort develops a reputation that amounts to a trade mark.

### COMMUNITY BREEDERS' ASSOCIATIONS

Probably no movement inaugurated by the Station other than the discovery of the Babcock test, has been so important in the development of dairying in Wisconsin, as the organiza-

tion throughout the state of these community centers for the breeding of high grade and pure bred dairy stock of one kind, and the encouraging of better methods of managing dairy herds. The Animal Husbandry department has given 18 lectures and 8 demonstrations this year under the auspices of those associations already organized. Fifteen new centers have been formed, making in all 62 that are now in operation in 35 counties of the state.

The older organizations are now deriving material benefit from collective advertising of their surplus stock. The Waukesha County Guernsey Breeders' Association this year in addition to private sales held three public sales at which over \$53,000 worth of stock was sold. Two other public sales held by one member aggregated over \$54,000 more. Sixty-six breeders in this one county alone now own over \$400,000 worth of Guernsey stock. It is no wonder that this region is known as the Guernsey Island of America.

#### HORSE BREEDING CLUBS

Dr. Alexander has pushed his campaign for the betterment of the Wisconsin horse industry by the organization of 10 county clubs in various portions of the state, the primary purpose of which is to foster improved breeding. The largest of these clubs is in Dane county, where some 257 members have joined. The clubs have been found to be helpful in the enforcement of the stallion enrollment law, as complaints of individuals are now made to the club officers whose duty it is to report to the department any infractions of the law.

Dr. Alexander has given 86 extension lectures on horse breeding and allied subjects during the past year.

#### COMMUNITY POTATO GROWING

Professor Milward of the Horticultural department has continued this year the work started last season of organizing the potato industry of the state with reference to growing standard varieties. The stock as it is now put on the market is so mixed in type that the reputation of the state for seed production has been considerably impaired. The excellent results that have arisen from community effort in the breeding of certain definite

types of livestock and pedigree grains in any one region can readily be duplicated in potato culture, and Professor Milward has been able to arouse a widespread interest in this matter. This year over one thousand bushels of pure seed have been grown by the Station at Conrath, Wausaukee, Spooner, and Waupaca, that will be used next season for dissemination purposes.

A large number of meetings have been held in connection with this movement, more especially this fall when the potato demonstration car was run in cooperation with the Soo railroad from October 26 till November 12.

Following this special train, a convention was held at Waupaca at which were gathered not only the potato growers from this vicinity, but from the northern and central parts of the state. As an outcome of this meeting, a State Potato Growers' Association was organized. Professor Milward has been very successful in awakening a keen interest in this movement.

Wisconsin already stands at the top so far as production is concerned, having a reported yield for this season of thirty-two million bushels, but with the millions of acres of land in the central and northern portions of the state that are especially adapted to the growth of this crop, it is important that this industry be guided along the lines of greatest profit from the standpoint of permanent agriculture.

#### COOPERATIVE SILO BUILDING CIRCUITS

An increasing demand continues to develop for the loan of the forms from the Agricultural Engineering department with which to construct solid wall concrete silos. In spite of the wet weather which prevailed this past season, 22 silos were built in twelve different towns by the use of these forms. At a nominal rental (\$7 to \$10, depending upon the number of farmers cooperating in the circuit) these forms are sent to a community with an instructor to show exactly how to erect the first silo. The forms are built for 12, 14 and 16 feet silos. A much larger number of silos could be erected if the farmers would take up the matter of silo building earlier in the season. The demand in late summer is such that 20 or 30 sets could be kept in the field, but manifestly, it is impossible to give unlimited accommodation in this way. The Station has available eight sets for this use.



### DISTRIBUTION OF BUILDING PLANS

Architects as a rule do not specialize in farm problems. The result is that most farm structures are built without adequate planning although to follow this method with such permanent improvements as buildings often produces regrettable results. The department of Agricultural Engineering has for several years aided applicants by sending out at a nominal charge standard plans (blue prints) of silos, barns, hog houses, ventilating systems, and other barn and stable equipment. During this past year over 4,300 such plans have been sent to farmers on request.

### FARM LITERATURE FOR RURAL SCHOOLS

The demand for bulletins and other published material for use in rural schools continues to grow apace. The rural teachers, required by law to teach agriculture in their curriculum of study, are eager to embrace the opportunity of securing information that they can use in connection with their classes.

Cooperating with the county superintendents, Professor Hatch has sent out this year 110,000 copies of circulars, bulletins, and mimeographed matter to satisfy such demand. In a not inconsiderable number of cases this demand on the part of teachers has entirely exhausted our supply of bulletins.

### BUTTER AND CHEESE SCORING EXHIBITIONS

The educational work in butter and cheese scoring exhibitions has been continued under the direction of Professor Lee of the Dairy department. Cooperating with him in the scoring of the products have been H. C. Larson of the State Dairy and Food Commission and various butter and cheese dealers.

One thousand three hundred and fifty exhibits, of which 997 were of butter, have been sent in this year for scoring by 279 factory operators. Since the inauguration of this work in 1907, the character of the creamery business has undergone radical changes. In 1907, 29% of the butter submitted came from whole milk factories; this past year, only 12.7% was made under these conditions. Yet, some of the exhibitors have kept up this work almost without interruption, and have profited materially thereby. Several factorymen have written saying that on account of the record they have made in the

scoring exhibits, they have been able to contract their butter at higher than market prices.

### OFFICIAL TESTS OF DAIRY COWS

This work under Professor Woll has grown in recent years to large proportions. During the last year nearly 5,000 tests were made for 166 breeders in all parts of the state, about 1,500 cows having been "officially tested" in all. The tendency in this work is toward the conduct of yearly rather than short-time tests, a tendency which has received considerable impetus through the results obtained in the Wisconsin Dairy Cow Competition that has been conducted during the past two years. These authenticated records of dairy performance have proven of much value to the pure bred interests of our state and have aided greatly in establishing the reputation of Wisconsin herds.

### CREAM TESTING

The College is continually in receipt of requests to test milk and cream to settle differences between factory operators and patrons. When all interested parties sign a statement as to mode of sampling, the Dairy department has aided in this work. Three hundred and ninety-three such samples have been tested this year.

### DISTRIBUTION OF STARTERS

In connection with the butter and cheese scoring exhibitions, 237 pure culture starters have been distributed by the department of Agricultural Bacteriology.

## II. COUNTY AGRICULTURAL REPRESENTATIVE SYSTEM

A new and most important line of extension endeavor has been inaugurated this past year through the establishment of County Agricultural Representatives, who will become resident representatives of the College of Agriculture in the respective counties of the state. In the dissemination of agricultural instruction, the lecture course system has long been followed with success, but the needs of the times indicate that a more

personal relation is likely to prove more effective than where the instruction is confined exclusively to the lecture platform.

The name—County Agricultural Representative—rather than expert or specialist, is purposely chosen as it is not intended that these men shall pose as experts upon all things agricultural. They do stand, however, midway between the College and the man on the farm, who may desire to secure direct information relative to the problems which may arise. The purpose of this plan is that this representative will be of direct aid to the



FIGURE 26. BOYS' COURSE IN ONEIDA COUNTY

During the winter, the county agricultural representatives organize short courses in agriculture for the farm boys.

farmer, enabling him to establish a more productive and permanent system of agriculture. As such, he should become an economic factor in the county, but it should also be his chosen mission to awaken and develop the community spirit and help guide the social forces along the pathway of progress.

In addition to the direct aid which he is to give the farmers mainly during the summer months, he also has charge of the agricultural instruction in the county training school for the training of rural teachers. This connection with the educational work of the county is purposely made with the hope that the character of agricultural instruction may be improved over that which has heretofore obtained. The law requires that agriculture shall be taught in all of the county schools.

The training school provides teachers for these schools. If the character of the instruction to these teachers can be such as to stimulate the teacher and impart to her some of the enthusiasm which the county agricultural representative should possess, it is to be hoped that the work in the school may be connected with the problems of the farmer in a direct and vital way. During the winter season short courses in agriculture for the farm boy (who, in the main has already left the country school) are organized, as well as farmers' courses in connection with the Agricultural Extension service.

In this work the county defrays one-half of the salary and expenses of the representative, the balance being met from the University treasury.

The work of these county men is exceedingly varied and difficult, and the main problem will be to secure properly trained and qualified persons to carry on the work. This is essentially a "one man" proposition and as such, success or failure depends almost wholly on that one man. No material investment in lands, buildings, or elaborate equipment is needed for this work.

*Oneida County.* This system was put into operation last winter in Oneida county, and Mr. E. L. Luther appointed as County Agricultural Representative with headquarters at Rhinelander. Later in the season the work was organized in two more counties, Eau Claire and Barron, Messrs. G. R. Ingalls and F. D. Otis being located respectively at Eau Claire and Barron.

A summary statement of the activities of these representatives may indicate the complexity and difficulties that surround their problems.

Mr. Luther was installed in his work in February, 1912, and was provided with very satisfactory office facilities in the court house adjoining the county training school. His first duties were in connection with a class of 15 teachers in the training school. At the same time he ran a 10-week short course in agriculture for 17 boys. In March with the aid secured from the college staff, a Farmers' Course was held at which 20% of all the farmers of the county participated.

Oneida is a new and comparatively undeveloped county with but three per cent of its land under cultivation. The preliminary survey he undertook soon showed in general that an acid condition of the soil obtained which made legume culture

difficult, a lack of crop rotation was also manifest, and but little dairy development had taken place. The problems that immediately confronted him were the introduction of improved live stock, liming to correct soil acidity, the growth of legumes to increase the nitrogen content of the soil and furnish forage for live stock, and the construction of silos as an adjunct to dairying.

From every possible angle these problems have been approached—through the teachers who have gone out into the rural schools; through the Boys' Short Course which has made possible the direct entrance into the farm home; through the Farmers' Course directly, where many of the mature farmers were reached; through the demonstration plots, which the representative has maintained on the county fair grounds; and through cooperative liming tests with sixty-four farmers by means of which practically a hundred successful plots of clover and alfalfa have now been established. By means of meetings in churches and school houses, the organization of farmers' clubs, live stock and grain associations, and the dissemination of timely short articles through the local press, the representative has stimulated the agricultural development of the county in a way that means not merely economic growth, but rural betterment.

*Eau Claire County.* Mr. Ingalls began his work in April in Eau Claire county. The problems of pioneer agriculture do not obtain so strenuously here as in the new North. The dairy industry is already well established, but the problem is the "boarder cow," that is eating up the profits of the rest of the herd. Through the medium of the county school, he has gotten the boys and girls of the farm interested in practical problems of arithmetic, such as determining the yield and profit of the individual cows of the herd. At the school or in the local cheese factory or creamery, the proper tests are made. In this way each farmer gets at the kernel of his problem without waiting for his neighbors to join hands and form a cow testing association. In eight months from the time he began his work in the county a total of 477 cows in 32 herds have been placed under test.

To aid the representatives in covering their territory more thoroughly, they have been provided in two cases with motor cycles. These have proven very serviceable where roads are

suitable, but in sandy sections travel with these has proven difficult. It has made possible a wider operating radius than is likely to obtain except along the railroad lines.

As Mr. Otis did not assume his duties in Barron county until August of this summer, his work does not properly come within the limits of this report.

In five months' time, two of these representatives have held 27 meetings with an attendance of 2,700 persons. Over 400 letters have been written and 340 farmers have visited the office for individual help. In 28 cases farmers have asked the representative to go out to their farms and 170 visits have been made on the initiative of the representative.

Since the inauguration of this system, this idea has spread rapidly through the state. Already over a dozen counties have signified their desire to enter into a similar relation with the College.

### III. EXTENSION INSTRUCTION

The collective extension activities that are carried on mainly during the winter months are supplementary to the field work that is done in summer. This instruction is given wholly by members of the Agricultural College staff, under the supervision of Professor Hatch, who acts as Secretary of the Agricultural Extension Service.

#### EDUCATIONAL TRAINS

This year several departments of the College have carried on extension work through the medium of educational trains that have proven unusually successful. These trains have in all cases been equipped at the expense of the various railroads, and have been run by the College alone, or in connection with various organizations, state and otherwise, that are interested in agricultural development.

*Seed Special.* In cooperation with the Crop Improvement Committee of the North American Council of Grain Exchanges and the Wisconsin Experiment Association, the Agronomy department ran a pure bred seed car over the C. M. & St. P. road last winter from January 15 to 26, inclusive, through the



At the close of the harvesting season, another potato train was run by the Horticultural department in conjunction with the Soo line system. The exhibit car started at Conrath in Rusk county on October 26, went west over this system, north on the new Frederic branch, then east to Rhinelander. From here it worked back to the Ashland division, going south from Prentice through Stevens Point, Hancock, Plainfield, and closing at Waupaca on November 12. At a number of the meetings the attendance ranged from 150 to 250 persons. This special was closed with a successful potato convention at Waupaca at which 10 members of the Station staff and other experts participated.



FIGURE 28. INTERIOR VIEW OF PURE BRED SEED CAR

This train was run early in the spring when the farmer was thinking about the grain he was to use for his seeding.

*Live Stock Specials.* Working in cooperation with the State Live Stock Breeders' Association, different educational trains have been run this season over the North Western, the St. Paul, the Omaha, and the Soo lines. These were started in the spring in the more populous southern counties, but requests



soon came in for similar trains over the more northern roads. These trains were met at almost every scheduled stop by hundreds of farmers who were greatly interested in the exhibition of improved live stock and appliances, on display in the exhibition car. Special efforts were made to demonstrate the waste and actual loss which follows the growing of poor quality of live stock of a scrub character in contradistinction to the returns secured from animals of selected breeding. The first train was run over the North Western line in the western part of the state from Lancaster to Mt. Horeb and from Lodi to Galesville. Eleven stops were made in a week from March 25 to 30, inclusive.



FIGURE 29. EXHIBIT CAR OF THE POTATO SPECIAL

Wisconsin this year excels all other states in potato production. This product would be greatly enhanced in value, if growers would follow the methods of live stock men and grow only pure strains of one or two varieties in a community.

The next week from April 1 to 6, the same exhibit was run over the St. Paul system from Milton to Fond du Lac, Portage, and Watertown, making ten stops.

Later in the season (June 3-8) another train was equipped and sent over the North Western in the eastern part of the state, and the Soo lines, for a week each. The trip on the North Western started in June at New London, ran to Rhinelander,

**LIVE STOCK "SPECIALS"**  
**1912**

**N.W. & OMAHA LINES** —○—  
**C.M. & ST. P.** - - -○- - -  
**S.O.** .....○.....

**NUMBERS INDICATE ATTENDANCE**

Map showing live stock "specials" for 1912 in Iowa. The map includes numerous towns and their corresponding attendance figures. The legend indicates three types of lines: N.W. & Omaha Lines (solid line with circles), C.M. & St. P. (dashed line with circles), and S.O. (dotted line with circles). Attendance figures are placed near the towns. The map shows a network of lines connecting various locations across the state, with attendance numbers ranging from 50 to 5000.

27,820 people in 67 localities in 38 counties saw good examples of improved live stock through these educational trains that were run in cooperation with the State Live Stock Breeders' Association. The attendance at each stopping point is indicated.

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main line. Twenty-three stops were made in 15 counties, with 9,300 people in attendance.

The total attendance on these various educational trains this season has been as follows:

Type of train.	Road	Dates covered	No. of stops	Attendance
Seed Special.....	St. Paul.....	January 15-25	11	1,845
Potato car.....	Soo.....	March 26-27	4	640
Live Stock Special.....	North Western.....	March 25-30	11	3,970
Live Stock Special.....	St. Paul.....	April 1-6	10	1,850
Live Stock Special.....	North Western.....	June 3-8	12	4,350
Live Stock Special.....	Soo.....	June 10-15	11	8,150
Live Stock Special.....	Omaha.....	August 18-30	23	9,300
Potato Special.....	Soo.....	Oct. 26-Nov. 12	21	2,120
			103	32,225



FIGURE 31. "MORE AND BETTER LIVE STOCK" EDUCATIONAL TRAIN

Farmers will attend meetings if they can see something worth while. Compare this system with the chart method for effectiveness in presentation.

These trains have covered in the aggregate over forty counties of the state and thirteen weeks of actual road work have been required to hold these meetings besides the no inconsiderable amount of time required to collect and install these exhibits. The various organizations and the railroads have co-operated most loyally to make this new movement as successful as possible.

The experience of this season has shown the value of this work. Much greater interest has been manifest where the work was confined to a single purpose and the value of this

method of campaigning is rendered more effective by a close follow-up campaign. Experience also demonstrates that better results are to be secured in the smaller towns where it is possible to get in closer contact with the farming population than where stops are made in cities of considerable size.

#### FARMERS' INSTITUTES

No material change in the nature of institute work has been made for several years. This season Superintendent McKerrow has held 134 institutes and 41 cooking schools, with a reported attendance of 122,000. This work is maintained on the basis of a separate fund of \$20,000.

#### FARMERS' COURSES

With the expansion of other lines of extension teaching, it was only possible this last year to hold eight farmers' courses. Four of these were held in connection with the County Schools of Agriculture. The attendance at Winneconne in Winnebago county and Onalaska in La Crosse county was substantially 2000 persons each, a number nearly as large as was in attendance at Madison. For the past two years the course at the University has been held in conjunction with the Wisconsin Live Stock Breeders' Association and the State Board of Agriculture. Courses were also held at Bayfield, in cooperation with the Commercial Club, at Plymouth, in connection with the agricultural work of the high school, and at Rhinelander with the newly established county agricultural representative. Eighty-one hundred persons were in attendance upon these eight courses. A much larger number of courses are requested, but with the limited force available this number cannot be materially increased.

#### COUNTRY LIFE CONFERENCE

In connection with the Farmers' Course which is held at Madison, a conference has been held for the past two years for those interested in rural affairs. This has been most successful and has been attended by teachers, bankers, and business men, and religious and social workers, as well as by the most progressive farmers of the state. At this conference the attempt has been made to stimulate the development of country leader-

ship from the ranks of the country itself by presenting the results which have been actually accomplished under such conditions. The success of these gatherings has led to the publication of a report of the proceedings which has been distributed to interested individuals.

In connection with the Summer School of Religion which is held under the auspices of the University Pastors' Association during the summer session of the University, different members of the Agricultural College staff have given a series of demonstrations to members of this school. As this Conference is composed mainly of country ministers, it has thus been possible to exert a definite influence on those primarily interested in rural improvement.

#### FARMERS' SCHOOLS

Nine one-week schools for definite instruction were given last winter at which there was a registered attendance of 383 who paid \$1.00 each. In these schools intensive instruction in two subjects, relating to agriculture or home economics, was presented. This work is thus much more highly specialized than the work of the farmers' institutes or farmers' courses. As it consists of actual laboratory operations, the number participating in any school cannot well exceed a fair sized class, but the instructors engaged in this work regard it as a most important phase of extension service, as judged by the actual results noted.

#### SCHOOL GARDEN WORK

The school garden work with children has been continued at the University by Prof. J. G. Moore and Prof. K. L. Hatch this summer, as before, to gain more extensive data on the subject of vacation gardens. The capacity of our plots for this work is always taxed to the limit and there are young people on the waiting list eager for a chance to take the place of any who drop out.

#### AGRICULTURAL PRESS SERVICE

The preparation of accurate, yet popularly written matter with reference to agricultural advancement, is one of our most important aspects of extension activity. Through the medium



of the University Press Bulletin, which reaches 700 newspapers and periodicals each week, a large amount of agricultural matter is presented. Two columns weekly have been published by the Western Newspaper Union for distribution through the county newspapers of the state, in addition to which many special articles are sent directly to specific papers. Through this service, nearly all of the farmers in the state are able to secure in their home paper the practical results of the experimental work carried on at the Station.



FIGURE 32. SCHOOL GARDENS AT AGRICULTURAL COLLEGE

Under the stimulus of competition, the children's interest is more readily awakened than when they work singly at home.

#### LECTURES AND CORRESPONDENCE

Over 200 individual lectures were given last year by various members of the Station staff before breeders' meetings, farmers' clubs, institutes, women's clubs, and schools.

A heavy and growing correspondence is carried on by the Station staff in reply to inquiries relative to various phases of agricultural practice. Some idea of the burden of this work can be obtained when it is known that the correspondence of the College now averages approximately 175 letters per day.

## EDUCATIONAL EXHIBITS

The annual exhibit of the College at the State Fair has gradually been improved year by year, until it is now recognized as one of the leading features of the Fair. It is housed by itself in a commodious building, and includes illustrative material to represent the manifold activities of the several departments. Efforts have been made to concentrate on one, or at most, a few

## IN WHICH CLASS ARE YOUR COWS?



FIGURE 33. ONE OF THE GRAPHIC COLLEGE EXHIBITS AT STATE FAIR

When each department concentrates its efforts in presenting only one or two lessons, he who walks past cannot avoid being taught. The pictures show a poor, a good, and an exceptionally good dairy cow, producing the amounts of butter shown in the piles—120 lbs., 360 lbs., and 800 lbs., respectively.

lines for each department, so as to emphasize the lesson that it was desired to teach. This educational exhibit furnishes a most valuable opportunity for bringing the work of the College vividly to the attention of many thousands of people—some of whom would be reached in no other manner.

An exhibit along dairy lines was also installed in November at the International Dairy Show in Milwaukee.

## INSPECTION SERVICE

In addition to its extension activities, the Experiment Station is required by law to supervise certain of the police activities of the state, such as the control of licenses for feeding stuffs (concentrated feeds for animals) and fertilizers, the inspection of field seeds for purity, the enrollment of stallions for public service, and the inspection of orchards and nurseries for noxious insects and plant diseases.

### SEED INSPECTION

Year by year, the inspection of field seeds as to purity and germination, under Professor Stone of the Agronomy department, continues to increase. This year 2,167 samples were tested in comparison with 1,238 for the preceding year. These samples come in the main from dealers who wish to safeguard their purchases. Eighty samples were collected from the open market and analyzed. It is impossible though, for the Station to safeguard this work properly, as the only fund available is the small fee of 25 cents paid for each analysis.

The receipts from this work are barely sufficient to cover the cost of mere examination and correspondence. Before this service can be placed on a thoroughly efficient basis, it must be recognized so as to permit of a field collector being kept quite continuously in the field collecting samples, under conditions which would enable prosecutions to be made for unlawful sale of seeds that are untested. It is through the medium of seed purchased that especially favorable opportunity is had for the introduction of noxious weeds of different kinds.

### FEED AND FERTILIZER CONTROL

The work in this division has been continued this past year on substantially the same basis as the year before. During the year, 41 brands of commercial fertilizers have been analyzed and licensed, and 239 manufacturers of concentrated feeding stuffs have had their various products licensed for sale. Nine hundred and twenty-eight dealers in 307 towns have been inspected during the year, and 981 feed samples analyzed in the laboratory. Professor Woll, who has charge of this division of the control service, reports that the quality of the concentrated



feeding stuffs sold in the state is gradually improving. Deficiencies in guarantees of valuable food components are becoming fewer and of less importance than in earlier years, and the feed manufacturers and dealers in general heartily cooperate in the enforcement of the law.

### STALLION ENROLLMENT

Under the operation of the stallion enrollment law, a decrease still continues in the percentage of grade as well as mongrel or scrub stallions used for public service in the state.

In 1907 when the first statistics were compiled by Dr. Alexander, who has had charge of this work since its inauguration, 65% of the stallions of the state were of grade or scrub breeding. In 1910 this percentage had fallen to 55.5%; in 1912 to 51.5%.

During 1912, 1,554 pure bred and 1,650 grade and scrub stallions had licenses in good standing; 256 new pure bred sires were also enrolled.

Pure bred sires have increased by 99 head in 37 counties, while grades have decreased by 66 in 26 counties. Mongrels are less by 125 in 47 counties; 194 grade sires and 190 scrubs have been retired from service during the year.

The financial statement of receipts and disbursements as required by law is as follows:

Receipts.		Disbursements.	
Fees, new licenses.....	\$1,106.10	Salaries .....	\$700.00
Fees, renewals .....	1,501.13	Clerical help .....	1,049.03
Fees, duplicates .....	68.50	Printing .....	91.56
Fees, transfers .....	225.00	Sundry supplies .....	13.54
		Traveling expenses .....	93.77
		Freight, express, messages..	6.20
		Postage, stationery, etc.....	205.75
		Balance .....	740.88
	<hr/>		<hr/>
	\$2,900.73		\$2,900.73

### NURSERY AND ORCHARD INSPECTION

Prof. J. G. Sanders, as chief nursery and orchard inspector, has inspected and licensed, in accordance with the state law, 147 nurseries, embracing 619 acres of nursery stock, and reported them free from dangerous pests. Nineteen Wisconsin dealers in nursery stock and the numerous agents selling nursery stock, have been licensed, in addition, and 57 nurseries out-

side the state have also secured licenses to sell at retail in Wisconsin.

Last year the San Jose scale, which is one of the most dreaded insect pests, was discovered at several points in the state, the most extensive infestation being found in Whitewater late last autumn. Immediate and thorough measures were at once taken to bring the pest under control throughout the state.

During the inspections exceptional interest is often manifested by nurserymen in discussions of the habits of certain

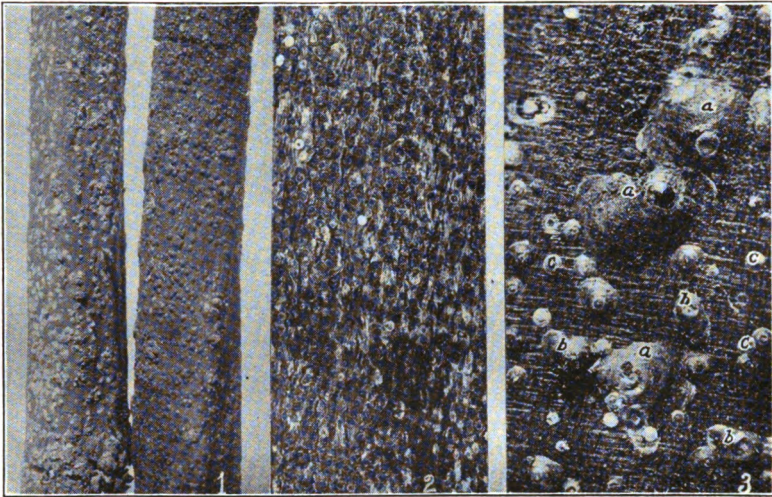


FIGURE 34. SAN JOSE SCALE, THE DREAD ORCHARD PEST

This insect pest has devastated many of the orchards in the East and in California. Through purchase of infested nursery stock, it has found its way into several places in this state. Only by the utmost vigilance has it now been brought under control. On left, scales natural size on apple twig. On right, scales on rose and apple, enlarged.

nursery pests and methods for their control. Many insects and diseases have been pointed out which under favorable conditions might become dangerous pests, and generally the growers were thoroughly interested, asking many questions not only regarding insects and diseases but also concerning general cultural methods. The various pests are frequently such powerful factors in determining success or failure in the nursery business or in fruit growing, that it is surprising so many of our growers have made no earnest effort to acquaint themselves with their horticultural foes.

In case of fire, flood, theft, or other destruction of stock, the owner would take immediate steps to prevent its repetition, but

since it is merely a recurring insect or fungus pest causing a 10 to 25% injury the loss is too often borne without much reasonable effort for control.

Professor Sanders urges all purchasers of nursery stock to observe the following precautions: Purchase stock only from reliable nurserymen, whose nurseries have been officially inspected. Ask to see the license of any nursery agent who offers to sell you stock. Do not pay high prices for any "new and wonderful fruits just imported" nor for any "new creations which are of great promise." The "strawberry-raspberry" is a "fake" which has been sold by many companies of supposedly high standing. The "Himalaya berry" is worthless in Wisconsin as a fruit producer. When in doubt regarding the varieties to buy for your region consult an authority in your neighborhood or write to the Horticulturist of the Experiment Station or to the Secretary of the State Horticultural Society.

Insist that your order is properly filled without unnecessary substitution of varieties. Reject all stock bearing distinct root or crown galls, and report the matter to the state inspector, sending samples of the diseased stock. Demand stock which has been grafted or budded on roots that are known to be hardy in your climate. Hundreds of trees of hardy varieties were winter killed in 1911-12 because the root system upon which they were growing was not hardy.

## ADMINISTRATION

The continued expansion of the different College activities has necessitated an increase in material facilities as well as staff. The more important of these additions are as follows:

### BUILDINGS

*Horticulture and Plant Pathology Building.* The new building for the departments of Horticulture and Plant Pathology, to which reference was made in the last report, and which has been in process of erection for two years, was completed and occupied in January, 1912. The Horticultural department occupies the basement and first floor, while the second floor has been fitted up for the Plant Pathology work. Already the growth of the latter work has necessitated the equipment of

space in the attic for the general class in plant pathology, while an additional greenhouse has been constructed this summer for the advanced research work.

*Crematory and Quarantine Building.* A one story brick building 24 x 38 ft., equipped with a crematory furnace, has been constructed at an expense of \$1,500 west of the farm buildings. This structure was designed as a detention or quarantine establishment as well as a crematory for the incineration of dead animals. For the present the building is in constant use in the manufacture of the hog cholera antiserum.



FIGURE 35. RESEARCH POULTRY PLANT

A south exposure with excellent drainage provides a favorable location for the experimental work of the poultry department.

*Research Poultry Plant.* To secure satisfactory results it was found desirable to separate completely the station poultry plant from the instructional work. This work has been developed this year on the Sandsten tract in close proximity to the farm house occupied by Professor Halpin. A very favorable site on the southern slope of the hill in close proximity to the new orchard is thus secured. The barn on the place has been remodeled and a cement floor installed so as to use the same as an incubator and brooding room. Fifteen colony houses with covered runs have been constructed for the breeding and physiological work now in progress.

*Verona Creamery.* With the growth of the city of Madison, more and more, the nearby milk supply which we have drawn on for the University Creamery is being curtailed. To main-

tain a supply for our experimental work and teaching, a creamery has been purchased at Verona, ten miles from the city, and the product there secured shipped to our University Creamery. As this creamery receives its supply directly from the farmers, it gives an opportunity to study problems under more natural conditions than at the University Creamery where a large proportion of cream received is collected by wagon.

*New Buildings Under Construction.* The Agricultural Chemistry and also the Home Economics buildings, which were started in the fall of 1911, are still in process of erection. These structures will probably not be completed before the year 1913-14. When finished they will afford much needed relief. The



FIGURE 36. DRIVE THROUGH EAGLE HEIGHTS FARM

Concrete posts properly made are practically indestructible. These are sufficiently reinforced to withstand any reasonable shock.

new department of Forestry organized this fall will be housed in the Chemistry building.

#### LANDS

The Eagle Heights farm of 156 acres, acquired last year, when purchased was in a badly run down condition. No outbuildings of any account existed and no fences to speak of on the



entire farm. The soil, through lack of fertilization (there having been no live stock kept on the place) has yielded only mediocre crops. Some of the fields have been in old timothy and blue grass sod for over a decade and the little crop produced removed in the form of hay.

The first problem is to restore the fertility of the place. Nothing as yet has been attempted in the matter of buildings, but several of the fields have been enclosed by the construction of permanent division fences in which the posts are made out of reinforced concrete as shown in Figure 36.

#### BRANCH EXPERIMENT STATIONS

Reference was made in last report to the fact that three branch experiment stations had been established in northern and central parts of the state, viz.,

1. Spooner, Washburn County, on the sandy, jack pine soils.
2. Ashland Junction, Bayfield county, on the Superior red clay.
3. Marshfield, Wood county, on the Colby silt loam.

*Marshfield Station.* The first two were organized during the preceding year, while the Marshfield Station has been started this year. Eighty acres of land were purchased by Wood county and the city of Marshfield and donated to the Regents for this purpose. Some of this land had been under cultivation for a number of years and is therefore well suited to the needs of the Soils department in studying the effect of cropping on fertility. Especial attention will doubtless also be given to the effect of drainage on these close textured soils. This soil is a fine silt loam, known as the Colby silt loam, that is characteristic of about 5,000 square miles of the north central part of the state and also resembles closely the Kennan clay loam of which there are about 7,000 square miles. This station therefore represents in a general way the dominant soil type embraced in a region bounded roughly by Marshfield, Merrill, and Medford.

*Ashland Station.* Considerable building and land clearing has been done this year on the Ashland farm. An office building 16 by 36 feet, two stories high with a fire proof vault, also a foreman's cottage, have been built (see frontispiece). These buildings stand on a sightly knoll in close proximity to both the Northern Pacific and the Omaha railroads at Ashland Junction,

and with their moss green shingles and plaster exterior, present a striking appearance from all passing trains. These two structures cost \$3,000. A machinery shed has also been built this season.

Nearly sixty acres of this cut over tract has been cleared and broken this year, some crops being grown. Within little more than a year this wild land has been converted from brush and old pine stumps into a well equipped station for the further prosecution of experimental work to aid in developing the agricultural resources of this red clay region.

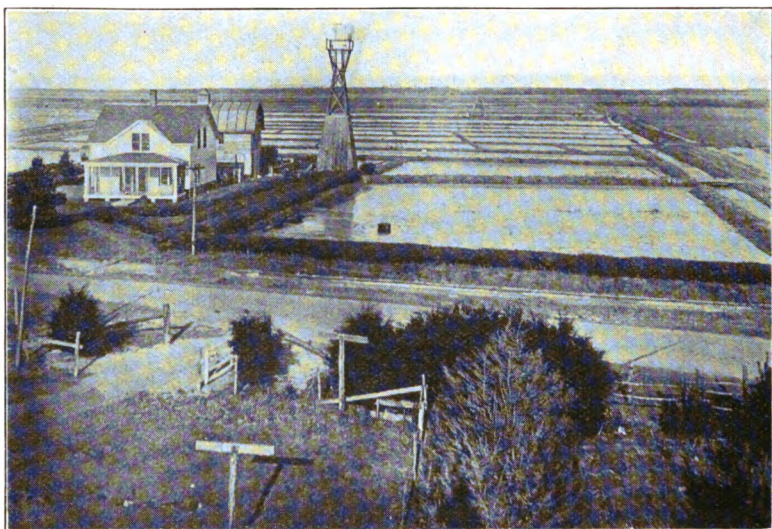


FIGURE 37. CRANBERRY STATION NEAR GRAND RAPIDS

Each year the benefits from sanded bogs and improved methods of culture are demonstrated on these plots. View shows bog flooded for winter protection.

*Spooner Station.* Additional barn space has been constructed on this station this year, making it possible to house a small dairy herd. On these light sandy soils live stock is a desirable adjunct to furnish manure, and part of the land is being built up by the use of fertilizer in this way, while on other fields green manuring is practiced.

Clearing operations have been continued on this farm this season, the larger part of the north eighty (except wind strips) having been cleared and broken. A cottage for the foreman is in process of erection this fall.

*Cranberry Station, near Grand Rapids.* The building hereto-

fore occupied as a laboratory has been increased in size to serve as a residence (during the summer months) for the Superintendent of this work. Considerable replanting and conversion of wild into the subjugated sanded bog has been made this season.

### DEMONSTRATION STATIONS

In accordance with the law passed in 1911 (Chapter 624, Laws of 1911) authorizing the establishment of county demonstration stations, the first station to be organized under this act was established this last year in Douglas county within the city limits of Superior. A tract of 20 acres was leased by the county board for a period of five years with the privilege of extending the same for another five year period. This tract is a typical Superior red clay and will be used to demonstrate the results which have been worked out as applicable to this soil type. A combined office and storage structure 24 x 36 ft. has been erected on this station at a cost of \$450.

The second demonstration station has just been located by the Regents this fall in Rusk county, on the Kennan clay soil type. These demonstration stations are jointly supported by specific appropriations made by the county and the state.

### PUBLICATIONS

Much of the work of the Experiment Station in progress at any time may not be put into final form for publication in the Bulletins or Research Bulletins for some time. Therefore, if any results are secured which are of direct and immediate practical value, the tentative conclusions are generally made known without delay through the medium of the press service. In some cases technical and purely scientific results are presented in various scientific journals of recognized standing, so as to bring the results of experiment station endeavor more prominently before scientific men in general, to whom the experiment station publications may not be available.

During the past year the following articles have been contributed to scientific journals by various members of the staff:



- The Unattached Aecial Rorms of Plant Rusts in North America—  
A. G. Johnson, Proc. Indiana Acad. Sci., 1911, 375-413.
- Investigations of the Potato Fungus, *Phytophthora infestans*—L. R.  
Jones, U. S. Dept. Agr., Bur. Plant Indus., Bul. 245.
- Culturing of Parasitic Fungi on the Living Host—I. E. Melhus, Phy-  
topathology, **2**, 195-203.
- On the Relation of Body Weights of Dairy Cows to Their Produc-  
tion—F. W. Woll, Proc. Soc. for Prom. Agr. Sci., Rpt. 33 (in  
press).
- Paraffin Blocks for Growing Seedlings in Liquid Culture Solution—  
Conrad Hoffmann, Centbl. Bakt., **34**, Abt. 2, 430.
- A Contribution to the Subject of Soil Bacteriological Analytical  
Methods—Conrad Hoffmann, Centbl. Bakt., **34**, Abt. 2, 385.
- The Protein and Phosphorus Content of Azotobacter Cells—Conrad  
Hoffmann, Centbl. Bakt. (in press).
- Results with the Complement Fixation Test in the Diagnosis of Con-  
tagious Abortion in Cattle—F. B. Hadley and B. A. Beach,  
Amer. Vet. Review, **42**, 43-51.
- Volatile Fatty Acids and Alcohol in Corn Silage—E. B. Hart and  
J. J. Willaman, Jour. Amer. Chem., Soc., **34**, 1619-1625.
- Quantitative Determination of Benzoic, Hippuric, and Phenaceturic.  
Acids in Urine—H. Steenbock, Jour. Biol. Chem., **11**, 201-209.
- Studies on the Factors Concerned in the Ripening of Cheddar  
Cheese—E. G. Hastings, A. C. Evans, and E. B. Hart, Centbl.  
Bakt., (in press).
- Notes on the Creatinine Excretion of the Pig—E. V. McCollum,  
Amer. Jour. Physiol., **29**, 210-214.
- Nature of the Repair Processes in Protein Metabolism—E. V. McCol-  
lum, Amer. Jour. Physiol., **29**, 215-237.
- Comparative Efficiency for Growth of the Total Nitrogen from Alfalfa  
Hay and Corn Grain—E. B. Hart, G. C. Humphrey, and F. B.  
Morrison, Jour. Biol. Chem., **12**, 133-153.
- On the Creatine Metabolism of the Growing Pig—E. V. McCollum  
and H. Steenbock, Jour. Biol. Chem., **13**, 209-218.
- Synthesis of Lecithin in the Hen and the Character of Lecithins Pro-  
duced—E. V. McCollum, J. G. Halpin, and H. A. Drescher, Jour.  
Biol. Chem., **13**, 219-224.
- A Method of Recording Types and Variations in Fruits and Vege-  
tables by Direct Printing—O. G. Malde, Amer. Breeders' Maga-  
zine, **3**, 52-56.
- A Case of Sex-Linked Inheritance in the Domestic Pigeon—L. J. Cole,  
Science, **36**, 190.

## EXPERIMENT STATION PUBLICATIONS

With reference to the regular publications of the Station, nine bulletins, embodying the more practical aspects of the Station experiments, six research bulletins of a technical character, and twelve circulars of information, giving specific agricultural information and results of our regular inspection service, have been published.

A brief digest of the material published during the year is here presented to show the nature and character of the publications of the Station.

## BULLETINS

**214. Concrete Silo Construction.** (Ocock and White) The high price of lumber has caused many Wisconsin farmers to turn to concrete for constructing silos. Simple directions are here given which will enable the farmer to do all of the work connected with the building of solid wall concrete silos, including the construction of inexpensive forms.

**215. Poultry House Construction.** (Halpin and Ocock) To answer the many inquiries continually received concerning poultry house construction, this bulletin has been prepared. Directions are given for erecting both portable colony houses and houses of the non-portable type, together with plans for interior fixtures, such as perches, dropping boards, nests, and watering devices.

**216. The Use of Explosives in Clearing Land.** (Kadonsky) One of the most important questions in connection with the development of upper Wisconsin is the removal of stumps. For this purpose explosives are at present largely employed. To determine the best methods of using explosives, investigations were conducted by the Wisconsin Experiment Station in cooperation with the United States Department of Agriculture and the Minnesota Experiment Station, the practical results of which are presented in this bulletin.

**217. Practical Lessons from the Management of the University Dairy Herd.** (Humphrey and Woll) In spite of the great increase in cost of feed, the cows in the University dairy herd make an average profit of over \$50 per year, and yet are given no special attention which any farmer could not duplicate. This bulletin shows the lessons to be learned from the record of the herd for the year, and presents data concerning questions which were studied, including the relative economy of high- and medium-protein rations, and the effect of early spring pasturage.

**218. Report of the Director.** (Russell) This, the twenty-eighth annual report of the Director of the Experiment Station presents briefly the work of the Station during the past year. The state of progress of the principal lines of research work is shown, and a brief report of the Extension Service is given. A brief digest of each publication issued during the year, is also included, together with the annual financial statement.

**219. Cranberry Bog Management in Wisconsin.** (Malde) To ensure the maximum returns from cranberry culture, proper bog management is absolutely essential, especially sanding and clean culture. Complete directions are given in this bulletin for the management of bogs, including the care of new plantings and bearing vines, destruction of weeds, fertilization, and protection from insects and frost.

**220. Better Cream Through Grading; A New Butter Moisture Test.** (Benkenendorf) Deterioration in the quality of creamery butter in the last few years is due largely to improper cleaning of hand separators and poor care of cream on the farm. This can be remedied only by grading the cream, as here de-

scribed, and paying higher prices for the better grades. A new moisture test for butter, which requires only five minutes for heating the butter, is also described.

**221. *Getting the Most Profit from Farm Manure.*** (Hart) At least \$25,000,000 worth of fertilizing constituents is lost annually from Wisconsin farms through careless and improper handling of the farm manure. Such losses can be prevented by the methods of management here described.

**222. *Crop Rotation for Northern Wisconsin.*** (Delwiche) Now that upper Wisconsin is being developed so rapidly, it is imperative that suitable cropping methods be adopted for that section of the state. This bulletin gives the results of experiments conducted at the Branch Stations as well as observations on the work done by practical farmers.

**223. *The Climate of Wisconsin and Its Relation to Agriculture.*** (Whitson and Baker) Among the factors which influence the agriculture of a state, none is more important than climate. This bulletin, illustrated by numerous graphic maps and charts, discusses in detail the climatic conditions in the different sections of Wisconsin, explains how the climate is modified by such factors as latitude, altitude, and distance from large bodies of water, and shows the important relations which exist between the agriculture and the climate of the different sections.

**224. *Selecting Steers for Feeding.*** (Tormey) Though Wisconsin is primarily a dairy state, some sections are especially adapted to beef production. For men living in these localities, this bulletin presents helpful hints on selecting and buying feeders, describes the various market classes and grades of cattle, and gives suggestions concerning marketing the finished cattle.

## RESEARCH BULLETINS

**19. *Effect of Heat and Oxidation on the Phosphorus of the Soil.*** (Peterson) By oxidation of various soils with hydrogen peroxide about 90% of the organic matter was destroyed, and the solubility of the phosphorus in fifth-normal acid markedly increased. Heat did not increase the solubility so much, and caused no further increase after the action of hydrogen peroxide. A corresponding increase in solubility of iron and aluminum, indicated that the phosphorus, though set free by the oxidation of organic matter, may have come from aluminum and iron phosphates enclosed within the organic matter.

**20. *Factors Influencing the Availability of Rock Phosphates.*** (Truog) Laboratory experiments in which organic matter was composted with raw rock phosphate (floats) showed only a slight solvent action of the fermenting material on the phosphate. The author states, however, that since in such tests the dissolved substances are not removed as under field conditions, laboratory experiments fail to imitate field conditions with regard to a most vital consideration. In pot experiments with corn and oats grown on quartz sand, the availability of floats to the crop was increased to a marked degree by mixing it most thoroughly with the sand.

**21. *Studies of the Nutrition of the Pig.*** (McCollum and Steenbock) Included in this bulletin are three papers: Notes on the Creatinin Excretion of the Pig; Nature of the Repair Processes of Protein Metabolism, and A Metabolism Cage for the Pig. (1) When pigs were kept on a nitrogen-free diet of starch, inorganic salts, and water for 21 to 38 days, a nearly constant ratio of creatinin nitrogen to total nitrogen in the urine was reached, the creatinin nitrogen forming from 17.56 to 22.00 per cent of the total nitrogen. (2) The results of experiments in which pigs were fed zein, casein, and gelatin, as the sole source of protein, indicate that the repair processes of protein metabolism are of a different character from the growth processes. (3) The metabolism cage devised by the authors for the pig is described.

**22. *Metabolic Water; Its Production and Role in Vital Phenomena.*** (Babcock) This bulletin presents the results of studies covering several years on the production and functions in plant and animal tissues of metabolic water, i. e., water produced within the cell by respiration and other vital processes. It is shown that the production of this metabolic water insures a constant supply of water to all respiring cells, plays an important part in the germination of

seeds, is the chief source of succulence in ripened fruits, and is a leading factor in the development of sap pressure in plants. In the case of certain moths and weevils which live on air dry food, it is metabolic water for the most part which meets the needs of the animal.

23. *Relation of Soil Bacteria to Evaporation.* (Hoffmann) In tests both with normal soils and with sterilized soils inoculated with pure cultures of soil bacteria, bacterial activity increased the evaporation of water from the surface of the soil. This increased evaporation was evidently caused by the increase in the amount of soluble substances brought about through the activity of the soil organisms, and the consequent influence upon the surface tension, capillarity, and diffusion of the soil moisture.

24. *The Diagnosis of Contagious Abortion in Cattle by Means of the Complement Fixation Test.* (Hadley and Beach) The method of using the new complement fixation test for the diagnosis of contagious abortion is described in detail, and a few of the many instances are cited in which the diagnosis by means of the test was later substantiated by the history of the case. The methods of handling infected animals and of preventing infection in the herd are also briefly discussed.

### CIRCULARS OF INFORMATION

27. *How to Use the Babcock Test* (Sammis). Simple, illustrated directions for testing whole milk, skim milk, and cream by the Babcock test, including mixing and sampling and preserving the samples. The method of calculating factory dividends on the basis of the test is also shown.

28. *Distribution of Licensed Stallions in the Counties of Wisconsin During 1911.* (Alexander) This circular shows the progress in Wisconsin horse breeding brought about by the stallion enrollment law, and contains a complete directory of the owners of all stallions enrolled in the state, with the name and breed of the stallion. Census statistics regarding the horse industry, and the laws pertaining to horse breeding in Wisconsin are also given.

29. *A Method of Making a Social Survey of a Rural Community.* (Galpin) Complete directions are given for making a social survey, which the author defines as an attempt to photograph the community so as to show every home in all its social connections with all other homes in the community. Some of the results which may be expected from such a survey are also mentioned.

30. *Chemical Analyses of Licensed Commercial Feeding Stuffs, 1911.* (Woll) In this circular is presented the annual report of the inspections made under the State Feeding Stuffs Law. A list is presented of the 233 manufacturers or dealers who took out licenses for the sale of 966 different brands of feed, and the guaranteed and the actual composition of 929 samples of feeding stuffs analyzed during the year is given, together with a discussion of the various feeds.

31. *Commercial Feeding Stuffs and Fertilizers for Sale in Wisconsin, 1912.* (Woll). A list of the manufacturers of concentrated commercial feeding stuffs and of commercial fertilizers, who took out licenses in accordance with the State Feeding Stuffs Law, is here given, together with the names of the various brands of feed licensed.

32. *The "Coming of Age" of the Babcock Test.* (Russell) On the occasion of the twenty-first anniversary of the invention of the Babcock test, this circular chronicles the history of this test which saved the dairy industry from ruin, and sets forth the far-reaching results which have followed the universal adoption of the test.

33. *Analyses of Licensed Commercial Fertilizers, 1912.* (Woll) This circular gives a list of the commercial fertilizers licensed for sale in this state, with the names of the manufacturers and the guarantees for valuable fertilizer ingredients. The results of the analyses of these and other brands of fertilizers are also shown.

34. *Sewage Disposal for Rural Homes.* (Ocock and Wright) Complete instructions are given for laying out and installing septic tank systems of sewage disposal adapted to homes in rural communities, and the process by which the sewage is purified in such a system is explained.

35. *Importance of Alfalfa as a Wisconsin Forage Plant.* (Moore) This circular sets forth the merits for our state of alfalfa, the queen of forage plants, and gives simple directions for its culture, from the time the seed is purchased until the hay is placed in the mow.

36. *Potato Diseases in Wisconsin and Their Control.* (L. R. Jones) The potato is now one of our important crops and is destined to become even more important. However, preventable diseases and insect pests often seriously reduce the profits of growers who do not use proper control measures. This circular sets forth the symptoms and occurrence of the various diseases and insect pests, and explains the remedies and means of control.

37. *The Feed Unit System for Determining the Economy of Production of Dairy Cows.* (Woll) The feed unit system has for years been extensively used in north European countries for comparing the relative economy of production of different cows, but has not hitherto been fully described in this country. As in this system a simple definite figure, which is independent of the market values of feeds, expresses the total feed eaten by any cow, a valuable means is furnished for studying and comparing individual cows and herds.

38. *Wisconsin Bankers' Agricultural Contests.* (Moore and Hatch) These pure bred seed contests, conducted in cooperation by the Wisconsin Bankers' Association and the College of Agriculture, have proven not only a most successful means of disseminating pure bred seed, but have also been valuable in coordinating the mutual interests of the town and the country. This circular describes the method by which communities can secure one of these contests, and gives helpful suggestions for those undertaking the management of such an enterprise.

## FINANCIAL STATEMENT

*The Wisconsin Agricultural Experiment Station, in account with the United States appropriation.*

1911-1912.	Dr.	Cr.
To receipt from Treasurer of the United States as per appropriations for the year ending June 30, 1912, under the acts of Congress approved March 2, 1887, and March 16, 1906 .....	\$30,000 00	
By salaries .....		\$17,675 00
By labor .....		5,834 66
By freight and express .....		7 45
By chemical supplies .....		632 47
By seeds, plants and sundry supplies.....		1,085 30
By feeding stuffs .....		2,509 18
By fertilizers .....		6 78
By library .....		299 06
By tools, implements and machinery.....		47 70
By furniture and fixtures.....		14 48
By scientific apparatus .....		1,418 36
By live stock .....		442 06
By traveling expenses .....		34 50
	\$30,000 00	\$30,000 00

We, the undersigned, duly appointed auditors of the corporation, do hereby certify that we have examined the books and accounts of the Wisconsin Agricultural Experiment Station for the fiscal year ending June 30, 1912, that we have found the same well kept and classified as above, and that the receipts for the year from the treasurer of the United States are shown to have been \$30,000, and the corresponding disbursements \$30,000, for all of which proper vouchers are on file and have been by us examined and found correct.

And we further certify that the expenditures have been solely for the purpose set forth in the acts of Congress approved March 2, 1887, and March 16, 1906.

THEODORE M. HAMMOND,  
GILBERT E. SEAMAN.

Executive Committee.

Attest.

M. E. McCAFFREY,  
Secretary.

# The University of Wisconsin

## Agricultural Experiment Station

### STAFF

#### THE PRESIDENT OF THE UNIVERSITY

H. L. RUSSELL, Dean and Director  
F. B. MORRISON, Assistant to Dean

S. M. BABCOCK, Assistant Director  
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**PART II**

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**RESEARCH BULLETINS  
NUMBERS 19 TO 24 INCLUSIVE**



# Effect of Heat and Oxidation on the Phosphorus of the Soil

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BY P. P. PETERSON

## PREFATORY NOTE

It is a well known fact that virgin soils, as a rule, show a high degree of fertility. This fertility lasts for a variable length of time but in practically all cases when exhaustion does come, it is phosphorus alone or with other elements which has become lacking to the crop. Nevertheless, when a determination of the total amount of phosphorus in the virgin and cropped soils is made, it is always found that only a part, and often a small part, of the amount present in the virgin condition has been removed before the soil shows exhaustion. This is particularly true of soils having a large amount of organic matter such as marsh and black prairie soils. These facts suggest that the phosphorus in virgin soils which becomes available readily is in organic matter which is easily oxidized. To test this hypothesis Dr. A. F. McLeod, formerly of this Department, at the suggestion of the writer undertook the oxidation of virgin soils by the use of varying strengths of hydrogen peroxide with the thought that it would be possible by so doing to extract the phosphorus set free in such oxidation. It was found, however, that fixation occurred so rapidly that the phosphorus set free by oxidation could not be extracted. The investigation was later taken up by Dr. P. P. Peterson, and the results reported in the following pages secured.

A. R. WHITSON.

## INTRODUCTION

The important role which the phosphorus of the soil plays in crop production has long been known. In 1804 De Saussure, speaking of calcium phosphate which he had found in the ashes of plants, wrote as follows: "I have found this same salt in the ashes of all plants that I have investigated, and there is no reason to assert that they could exist without it." But little was written on the subject until about 1840 when Liebig and his contemporaries took it up with renewed interest, and from that time the interest has been kept up. As a result the complexity of the problem and the literature have grown apace. The question that De Saussure raised in the statement quoted above was a simple one and was soon settled in the affirmative. Plants, in general, do need phosphorus for their best growth. Boussingault took up the next question: "How much phosphorus does a crop remove from the soil?" It was soon discovered that different kinds of crops use different amounts of phosphorus. Magnus attempted to show that phosphorus is not a limiting factor in crop production. He showed that a crop of turnips removes so small an amount of phosphorus from the soil as to make it possible for them to be grown almost indefinitely on the same soil without exhausting the supply of phosphorus. Liebig took up the other side of the question and soon won the approval of the chemists by his arguments.

Among the questions which have arisen since the time of Liebig is that of the availability of the phosphorus of the soil to plants. Various methods have been devised for measuring the availability but none are thoroughly reliable. They give only a rough approximation. The one that has been used in this work is that developed by Stoddart,<sup>1</sup> the fifth normal nitric acid extraction method. While the theory upon which this method is based is doubtless faulty, it has been shown practically that when the solubility of phosphoric anhydride in fifth normal nitric acid is lower than .015 per cent the soil is likely to be deficient in available phosphorus. And since in this work an approximation is the best that can be expected, this method has been suitable.

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<sup>1</sup> Jour. of Ind. & Eng. Chem. 1, No. 2, p. 7

In Wisconsin there are many soils high in total phosphorus and yet deficient in available phosphorus when measured by its solubility in fifth normal nitric acid. Table I gives data for a number of soils with which we have worked that show this characteristic.

TABLE I SOME WISCONSIN SOILS HIGH IN TOTAL PHOSPHORUS BUT LOW IN AVAILABLE PHOSPHORUS

Lab. No.	Kind of Soil	Location	P <sub>2</sub> O <sub>5</sub> Sol- uble in N/5HNO <sub>3</sub> Per cent of soil	Total P <sub>2</sub> O <sub>5</sub> Per cent of soil
234	Virgin .....	St. Croix Co. ....	.013	.117
239	Cropped, well handled .....	Vernon Co. ....	.008	.154
283	Virgin .....	Grant Co. ....	.009	.175
296	Virgin .....	Richland Co. ....	.010	.149
485	Cropped, still considered to be in good state of fertility .....	Watertown .....	.014	.139
519	Virgin .....	Washington Co. ....	.012	.187
1288	Cropped (a) .....	Richland Co. ....	.003	.204
1371	Virgin .....	Waukesha Co. ....	.013	.121
3X	Cropped (a) .....	Jefferson Co. ....	.013	.067

(a) 1288 and 3X have been tested in pot experiments and are shown to be in need of a phosphate fertilizer. 1288 also responded readily to rock phosphate treatment in the field.

In considering this table, the following things should be noted. The soils have come from various parts of the state. Of the nine, five are virgin and four cropped. Therefore these do not represent a class of soils that have been exhausted by cropping. They have not been selected because of their acid or non-acid character.

The non-availability of so large a part of the total phosphorus of the soil caused us to carry on an investigation to determine if possible in what condition it existed to make it so useless to plants. There are many compounds of phosphorus which may be present in the soil. Some are inorganic and doubtless some organic compounds also are there. It is possible also that both phosphorus and iron or aluminum may be in combination in the same organic molecule. Eggertz,<sup>2</sup> working with the ammoniacal extract of a soil, found that it contained from .15 to 7.58 per cent of phosphorus and concluded that the phosphorus was chemically combined with carbon. Van Bemellen<sup>3</sup> found a phenomenon of absorption of phosphorus by the soil and believed that the phosphorus not chemically combined in minerals.

<sup>2</sup> Centbl. Agr. Chem. **18**, 75

<sup>3</sup> Landw. Vers. Stat. **37**, 347



was absorbed physically by a humate-silicate colloid, but not in chemical combination with carbon. Wiklund<sup>4</sup> brought evidence against Van Bemellen and Schmoeger<sup>5</sup> followed with his classical work, confirming Eggertz and Wiklund and concluding also that the organically bound phosphorus consisted mainly of nuclein. This conclusion he arrived at by heating a peat soil to 140°—160° C in an autoclave. This gave a large increase in phosphorus soluble in 12 per cent hydrochloric acid. Nuclein when heated in the same way splits off phosphoric acid. He also found a similar increase in the solubility of sulphur in both soil and nuclein after heating. This similarity between the soil phosphorus and soil sulphur on the one hand and nuclein phosphorus and nuclein sulphur on the other he interpreted to mean that the phosphorus was combined with organic matter in the form of nuclein. Aso<sup>6</sup> did the same experiments with a peat from Japan and found the same results except that the increase in sulphur obtained by heating the peat was not nearly so marked. They both found some lecithin in the peat but the amount was inconsiderable.

The method which Grandeau developed for the extraction of organic matter from the soil has been used to estimate the organically bound phosphorus. The precipitate which is thrown down by adding a strong acid to the ammonia solution contains phosphorus. Grandeau<sup>7</sup> considered this to be from organic sources. Nannes<sup>8</sup> showed that the filtrate also contained phosphorus. Out of the .166 per cent contained in the peat with which he worked, .057 per cent was found in the *matiere noire*, .039 per cent was in the filtrate. Ladd<sup>9</sup> estimated that on an average nearly half of the phosphorus of eight soils with which he worked was in organic form though this varied greatly for the individual soils. A question has been raised by Fraps as to the correctness of the conclusions of Nannes and Ladd. Fraps<sup>10</sup> finds that four per cent ammonia will dissolve aluminum and iron phosphates and thinks part of the phosphorus obtained by the Grandeau extraction

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<sup>4</sup> Landw. Jahrb. 20, 909

<sup>5</sup> Ber. der Deutsch. Chem. Ges. 26, 336

<sup>6</sup> Tokyo Col. of Agr. Bul. 6, 277.

<sup>7</sup> Compt. Rend. 98, 201

<sup>8</sup> Jahresberichte über Agrikulturchemie 42, 89

<sup>9</sup> N. Dak. Exp. Sta. Bul. 32, 310

<sup>10</sup> Am. Chem. Jour. 39, 204

is from this source. In a recent work<sup>11</sup> he estimated that in twenty-seven of the soils with which he worked, 51 per cent of the phosphorus extracted by 4 per cent ammonia after a treatment with 12 per cent hydrochloric acid is from inorganic sources.

Hopkins and Petite<sup>12</sup> suggest a method for estimating the organic phosphorus of a soil by the difference in total phosphorus in the surface and subsoil. Stewart,<sup>13</sup> working at the Illinois Experiment Station, brought confirmatory evidence of their view by comparing the amount of phosphorus in the organic form as determined by the various methods. In his work he found that by the method of calculation of Hopkins and Petite, 46 per cent of the total phosphorus was in organic combination. By the method of Nannes and Ladd he found 55 per cent, by the method of ignition 60 per cent and by the autoclave method of Schmoeger 66 per cent. Fraps points out the errors likely to influence the results in the three methods last named. In the cases of the method of Nannes and Ladd and the ignition method, he brought experimental evidence against them, but this is not true of the autoclave method. Beside the objections raised by Fraps to the conclusions of Stewart there seems to be another objection to the method of Hopkins and Petite. In many soils the difference in the total phosphorus is in favor of the subsoil. This is true in some of the soils with which they were working when they suggested the method. For these soils the method certainly can not be applicable. We must conclude therefore that the method is not reliable.

The "ignition soluble" method was used by Nagoaka.<sup>14</sup> He tried the solubility of the phosphoric acid in strong hydrochloric acid, water, ammonium nitrate, 5 per cent acetic acid, 1 per cent citric acid, 1 per cent oxalic acid, and a saturated solution of carbonic acid. In all except the water in which only a trace was soluble, he found a material increase in solubility by heating. He also<sup>15</sup> compared the increase in solubility of phosphoric acid obtained by ignition by autoclave heating at a pressure of three atmospheres, and by steaming in Koch's apparatus.

<sup>11</sup> Tex. Sta. Bul. 136, 24

<sup>12</sup> Ill. Sta. Bul. 125, 204

<sup>13</sup> Ill. Sta. Bul. 145

<sup>14</sup> Tokyo Col. of Agr. Bul. 4, 265

<sup>15</sup> Tokyo Col. of Agr. Bul. 4, 265

He found that autoclave heating gives a greater increase in the solubility than either of the others. Steaming in Koch's apparatus gave practically no increase. Stewart<sup>16</sup> compared this method with other methods and found a smaller increase by it than by the autoclave heating method. Fraps<sup>17</sup> criticizes the ignition method because ignition causes an increase in the solubility of the mineral phosphates which may be in the soil. The assertion that the phosphorus may come from minerals is certainly well founded.

König, Hasenbäumer and Grossmann<sup>18</sup> showed that phosphoric acid was made more soluble in pure and carbonic water by oxidizing the soil with hydrogen peroxide. They were able to destroy 70 per cent of the organic matter of the soil in this way using very strong hydrogen peroxide. McLeod<sup>19</sup> working in this laboratory, showed that most of the organic matter could be removed from soils by a comparatively weak solution of hydrogen peroxide. In the investigation reported in the following pages this method of decomposing the organic matter has been used together with a method of dry heat, also used by König. The increase in the solubility of the phosphoric acid by heating the soil may come from the mineral phosphates as Fraps<sup>20</sup> has pointed out, but the increase by oxidation can hardly be from that source, except it should be by removing from the mineral particles an envelope which protects them from the solvents. This possibility will be considered in the experimental part of the bulletin.

## EXPERIMENTAL PART

### SOLVENT ACTION OF N/5 NITRIC ACID ON WAVELLITE AND DUFRENITE BEFORE AND AFTER HEATING

The amount of phosphoric acid extracted by fifth normal nitric acid on raw and roasted wavellite and dufrenite was first tried to determine if the effect that König had found on the solubility of the phosphoric acid of the soil by dry heat could be due to the presence of these minerals in the soil. A sample of

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<sup>16</sup> Ill. Exp. Sta. Bul. 145

<sup>17</sup> Tex. Sta. Bul. 136, 29

<sup>18</sup> Landw. Vers.-Stat. **69**, 30

<sup>19</sup> Unpublished work

<sup>20</sup> Am. Chem. Jour. **39**, 204

each weighing 2.5 grams was heated in a drying oven to the temperature given in Table II. Each sample was then digested with 250 c. c. of fifth normal nitric acid and 100 c. c. taken for duplicate determinations of phosphoric acid, ( $P_2O_5$ ).<sup>21</sup> Table II shows that the phosphoric acid of wavellite is more solu-

TABLE II. EFFECT OF HEAT ON THE SOLUBILITY OF WAVELLITE AND DUFRENITE

Temp. C.	PER CENT OF TOTAL $P_2O_5$ SOLUBLE IN N/5 $HNO_3$	
	Wavellite	Dufrenite
Not heated.....	4.12	0.8
180°.....	54.9	.....
200°.....	49.0	1.08
240°.....	98.7	.....

ble after roasting than before. Dufrenite is only slightly increased in solubility at a temperature of 200°. The wavellite contained 18.79 per cent and the dufrenite 19.6 per cent of phosphoric acid.

#### SOLVENT ACTION OF N/5 NITRIC ACID ON THE PHOSPHORIC ACID OF THE SOIL BEFORE AND AFTER HEATING

For the experiment with soils, 25 grams was digested with 250 c. c. of fifth normal nitric acid and 100 c. c. of the extract taken for each of duplicate determinations of phosphoric acid. Because of the increased solubility of the organic matter after heating to a high temperature the extracts of the samples of soil heated above 130° were a dark brown. The organic matter was destroyed by treatment with bromine in alkaline solution until the residue left on evaporation was a pure white. After heating the residue to 120° C. for several hours to dehydrate the silica it was taken up with nitric acid and the phosphoric acid determined in the usual way. To avoid oxidation of the organic matter which might take place by the atmospheric oxygen at high temperatures the soil was heated in a vacuum obtained by a filter pump.

This operation was carried out as follows: The soil was placed in a round bottom flask stoppered by a one-hole rubber

<sup>21</sup> Throughout this article the term "phosphoric acid" will be used for "phosphorus pentoxide."

stopper through which passed a glass tube connected with a manometer and the filter pump. The pressure as read on the manometer varied between 10 and 15 mm. Heating was accomplished at temperatures up to 100° C. by means of a water bath and for the higher temperatures by a Rose metal bath. A clay loam soil from the same farm as 1288 and closely corresponding to it except in total phosphoric acid was used. The total phosphoric acid was .154 per cent. Table III gives the results obtained.

TABLE III EFFECT OF HEAT ON THE SOLUBILITY OF THE PHOSPHORIC ACID OF THE SOIL

Temp. C.	P <sub>2</sub> O <sub>5</sub> soluble in N/5 HNO <sub>3</sub> . Per cent of soil
Not heated.....	.0028
50°.....	.0032
100°.....	.0038
130°.....	.0073
160°.....	.0178
200°.....	.0550
240°.....	.0440

It should be noticed that heat has little effect upon the solubility of the phosphoric acid until a temperature considerably above 100°, the ordinary cooking temperature, has been reached. A comparison of Tables II and III shows a lack of similarity in the effect of heat upon wavellite and the phosphoric acid of the soil. With both, an increase takes place at about 160°, but this is not so marked in the soil as in wavellite. At 200° the difference is in the other direction. The solubility of the soil phosphorus reaches its maximum at this temperature, whereas the solubility of the wavellite is no higher than in that heated to only 160°. At 240° the wavellite becomes almost totally soluble.

#### EFFECT OF OXIDATION WITH HYDROGEN PEROXIDE ON THE SOLUBILITY OF THE PHOSPHORIC ACID OF THE SOIL IN N/5 NITRIC ACID

While there is this lack of similarity between the action of heat upon wavellite phosphoric acid and soil phosphoric acid, it is seen that the idea of Fraps<sup>22</sup> that the phosphoric acid comes

<sup>22</sup> Am. Chem. Jour. 39, 579

from the minerals still is very probable. But we have to reconcile this idea with the experimental results of König, Hasenbäumer, and Grossmann,<sup>23</sup> that oxidation by hydrogen peroxide sets free phosphoric acid from the soil. It is hardly to be expected that oxidation can set free phosphoric acid from the minerals. If it cannot do so and there are iron and aluminum phosphates in the soil, a second increase should take place if the soil is heated after the organic matter has been destroyed by hydrogen peroxide. Experiments were carried out to determine whether this was the case or not. Fifty grams of soil was weighed into a tared beaker, wet with 50 c. c. of water, and 5 c. c. of a 30 per cent solution of hydrogen peroxide added. The beaker was covered with a watch crystal to prevent loss by spattering. After the hydrogen peroxide was exhausted, 5 c. c. more was added until effervescence ceased. Then the beaker was placed on a boiling water bath when the reaction began again. This treatment was continued until the soil ceased to lose weight on successive additions of hydrogen peroxide and drying to constant weight at 100°. The difference between the weight of the beaker and soil at the beginning and end of the operation gave the loss in organic matter. The soil was then pulverized and thoroughly mixed and divided into equal portions. One portion was heated to 240°, the other was not. A third sample of 25 grams was heated without previous oxidation and a blank sample of the same weight was prepared by wetting and drying it just as the oxidized sample had been wet and dried. The four samples thus prepared were extracted with fifth normal nitric acid in the usual way. The extract was heated with bromine water to decompose the dissolved organic matter, and the phosphoric acid determined in aliquot parts of 100 c. c.

Soil No. 1286 is a virgin clay loam soil from Gotham. No. 1288 is a cropped sample corresponding closely to 1286 (See Table I.). No. 1363 is a cropped soil from a field adjoining the one from which 1288 was taken. Until about six years ago it was allowed to run down, but since that time has been well handled. No. 1175 is a cropped soil from Pierce County. It is a sandy loam rather low in total phosphoric acid but high in available phosphoric acid. No. 1176 is a virgin sample corresponding very closely to 1175. No. 3x was taken from a barrel

<sup>23</sup> Landw. Vers.-Stat. 69, 30

sample from Ft. Atkinson. It is a crop soil which responded to a pot test for phosphoric acid. No. 1365 is a virgin clay loam from Brookfield. No. 1371 is a virgin sandy loam from Genesee. No. 1374 is a virgin sand from Eagle.

Table IV gives the results obtained in this experiment.

TABLE IV SOLUBILITY OF  $P_2O_5$  IN N/5  $HNO_3$  BEFORE AND AFTER OXIDIZING AND HEATING TO  $230^\circ C$

Lab. No.	Total $P_2O_5$ Percent of soil (a)	$P_2O_5$ EXTRACTED BY N/5 $HNO_3$ PER CENT OF SOIL				Total organic matter. Per cent of soil	Organic matter removed by $H_2O_2$ Per cent of soil
		Before treatment	After heating to $230^\circ C$	After oxidizing with $H_2O_2$	After oxidizing and then heating to $230^\circ C$		
1286	.231	.012	.096	.130	.124	5.98	.....
1288	.204	.003	.039	.069	.079	3.54	.....
1364	.159	.004	.060	.068	.062	4.54	3.44
1175	.095	.024	.050	.059	.051	1.42	1.32
1176	.118	.034	.066	.074	.072	2.57	2.26
3x	.067	.013	.056	.062	.062	2.35	2.06
1365	.105	.020	.....	.064	.057	.....	2.54
1371	.121	.013	.....	.043	.047	.....	1.38
1374	.069	.012	.....	.033	.035	.....	0.60

(a) Determined by fusion with  $Na_2 CO_3$ .

In this table there are several things that are worthy of note. In the cases where the data are sufficient to calculate the percentage of the organic matter removed by hydrogen peroxide, on an average, nearly 90 per cent of the organic matter has been removed. König was able to remove only 70 per cent with a much stronger solution of hydrogen peroxide. A comparison of columns 3 and 4 shows that a very large increase in the soluble phosphoric acid is obtained by heat alone. But the differences between the figures in 3 and 4 are considerably smaller in every case than the differences between the figures in 3 and 5 which represent the increases obtained by oxidation alone. Perhaps the most notable thing in this table is that it shows for most of the soils a decrease in the solubility of the phosphoric acid rather than an increase on heating subsequent to oxidation. In only two cases is an increase shown and they are so small as to be within the limit of experimental error. Where a decrease is shown, it too is within the limit of experimental error. However, a decrease might be expected, for if ferric or aluminum hydrate or ferric or aluminum oxide is present it will be-

come much less easily soluble after being heated to  $230^{\circ}$  than it was before. It is then likely to hold occluded small particles of ferric or aluminum phosphate and thus prevent it from the action of the solvent. The sandy soils do not show as large an increase in the solubility of the phosphoric acid as the clays do, perhaps because of the smaller amount of organic matter which they contain. The average increase for fine clay and clay loam soils is 50 per cent of the total phosphorus. For the sands and sandy loams it is 31 per cent.

The fact that there is no increase in the solubility of the phosphoric acid on heating a soil from which the organic matter has been removed is of special interest here because of Fraps' work in which he concludes that much of the "ignition soluble" and "ammonia soluble" phosphoric acid comes from the mineral phosphates of aluminum and iron. We cannot say positively yet that there are no mineral phosphates in the soil to account for the increase in solubility of phosphorus on heating the soil, but the results point in that direction. In the soils with which we have worked there are no mineral phosphates which increase in solubility upon being heated to  $230^{\circ}$ . Before a broader conclusion can be drawn, further work will have to be done with a more varied class of soils and with other solvents.

#### INCREASE IN SOLUBLE PHOSPHORIC ACID AT DIFFERENT STAGES OF OXIDATION

From the facts at hand we could not be certain that all of the phosphorus held by the organic matter was released by the oxidation as carried out. To make this point more certain and to discover in what stage of oxidation the greater part of the phosphorus is set free, experiments were carried out with samples of soil oxidized with varying amounts of hydrogen peroxide. Samples of 25 grams of soil were weighed into six tared beakers. To each one was added 50 c. c. of water, and 5 c. c. of peroxide was added to each of five. When the oxidizing power of the hydrogen peroxide was exhausted one of the oxidized samples and the blank were evaporated to dryness and dried at  $100^{\circ}$  to constant weight. To each of the remaining four, 5 c. c. of hydrogen peroxide was added and allowed to exhaust itself. One was then dried to constant weight and the others treated



with 5 c. c. more peroxide. This was continued until oxidation of each of samples 2, 3, 4, 5, and 6 had been carried out with 5, 10, 15, 20, and 25 c. c. of hydrogen peroxide respectively. Thus five different stages of oxidation were obtained. The solubility of the phosphoric acid in fifth normal nitric acid was then determined in the usual way.

An increase of the iron content of the extract had been noticed in samples previously investigated. With these samples the iron in the fifth normal nitric acid extract was determined quantitatively by titrating against standard potassium permanganate after reduction by a Jones reductor. The results given are averages of duplicate determination. The aluminum oxide was determined by subtracting the weight of phosphoric acid and ferric oxide from the weight of precipitate obtained from the extract by addition of ammonium hydroxide. Table V gives the results with three soils. In two of the soils the solubility of calcium and manganese was determined.

TABLE V EFFECT OF DIFFERENT STAGES OF OXIDATION ON THE SOLUBILITY OF THE PHOSPHORUS OF THE SOIL

c.c. 30% H <sub>2</sub> O <sub>2</sub> used	Total org. matter Per cent of soil	Per cent of organic matter removed	P <sub>2</sub> O <sub>5</sub> soluble N/5 HNO <sub>3</sub> Per cent of soil	Fe <sub>2</sub> O <sub>3</sub> soluble in N/5 HNO <sub>3</sub> Per cent of soil	Al <sub>2</sub> O <sub>3</sub> soluble in N/5 HNO <sub>3</sub> Per cent of soil
Soil 2x					
Blank .....	3.54	.0	.004	.12	.35
5.....		.5	.012	.18	.43
10.....		.25	.057	.35	.64
15.....		.66	.073	.43	.75
20.....		.69	.085	.45	.65
25.....		.80	.076	.46	.86
Material Soluble in N/5 HNO <sub>3</sub> Per cent of Soil					
Org. matter removed Per cent of soil	P <sub>2</sub> O <sub>5</sub>	CaO	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>	Mn <sub>2</sub> O <sub>3</sub> 3 4
Soil 818					
.0	.034	.54	.11	.41	.22
.48	.061	.54	.15	.40	.25
1.52	.116	.54	.32	.34	.51
2.48	.142	.49	.45	.59	.21
3.20	.155	.50	.55	.62	.24
2.96	.161	.49	.55	.62	.24
Soil 979					
.0	.066	.32	.10	.04	.22
1.08	.119	.35	.24	.08	.22
1.63	.129	.33	.30	.08	.....
2.25	.144	.32	.52	.11	.23
2.52	.146	.38	.54	.....	.24
3.20	.143	.35	.54	.11	.24

This table shows that the early stages of oxidation set free much more phosphoric acid than the later stages. There

is not much of an increase after one-fourth to one-third of the organic matter has been removed. This is to be expected, for the early stages of oxidation make soluble a large amount of organic matter that is not really removed but only made soluble and remains with the soil when the water is evaporated. When the soil is extracted with nitric acid the organic matter that is soluble is extracted with it and is then decomposed by bromine. A considerable part of the phosphorus may still be bound chemically to carbon compounds until further oxidation by bromine in the nitric acid extract. The very last stages of oxidation set free no phosphoric acid at all. In soil 2x removal of 60 per cent of the organic matter gives just as much soluble phosphoric acid as the removal of 80 per cent does. The same is true of the other soils if we assume that the highest amount of organic matter removed is 80 per cent. It seems reasonable in the light of these facts to believe that a removal of the entire amount of organic matter will not increase the solubility of the phosphoric acid above that already obtained.

An interesting thing shown here also is the increased solubility of iron and aluminum in the different stages of oxidation. The increase in the soluble iron is roughly parallel to the increase in soluble phosphorus but not strictly proportional to it. This may be due to one of two conditions. The iron and phosphorus may be combined with the carbon or they may be combined with each other and not with carbon. In the latter case the protection from solution before oxidation must be by insoluble organic matter. The protection can be conceived as being due to a complex similar to the one Van Bemelien believed to exist in the soil. If iron phosphate is held from solution in this manner, it seems reasonable to assume that some of the compounds of aluminum, manganese and calcium should also be held from solution by the same insoluble complex. This is seen to be the case. The calcium and manganese remain constant in solubility, and the aluminum varies in a more irregular manner than the iron does, but still increases in every case. In soil 818 the amount of aluminum in the nitric acid solution is so large in comparison with the iron that it was very difficult to get a good basic acetate separation of the iron and aluminum from the manganese. And in this soil the greatest variation is found in both aluminum and manganese, though it is very

small with the manganese. The aluminum in soil 979 was determined directly by the phenyl hydrazine method of Hess and Campbell as modified by Allen.<sup>24</sup> The manganese was determined as the pyrophosphate. The results here seem more reliable for these two metals than in the case of soil 818, and here there is an increase in aluminum closely corresponding to the increase of phosphorus and iron. From these facts it seems most likely that the iron is not combined with the phosphorus and organic matter into a single chemical compound. And it makes it appear also that the phosphorus does not all come from organic matter but that some of it is present as phosphates of iron and aluminum which are soluble in the acid when the organic matter is decomposed. This leads to the idea that iron and aluminum phosphates are in so intimate a mixture with the insoluble organic matter as to protect them from the solvent. The calcium and manganese form no compounds that are held in this mixture.

#### DIFFERENCE IN THE ACTION OF HYDROGEN PEROXIDE ON THE SURFACE SOIL AND THE SUBSOIL

If the phosphoric acid set free by oxidation and heat comes from the organic matter, we should not expect the same behavior with the subsoil as with the surface soil. In the first place, there is not so much organic matter in the subsoil as in the surface soil. Furthermore, the minerals in the subsoil have never been subjected to the action of organic acids formed by decaying organic matter to the same extent that the surface soil has. The minerals of the subsoil are therefore likely to be more like the minerals of the original rock. If this is the case, oxidation should set free less phosphoric acid from the subsoil than from the surface soil. Heat after oxidation should have more effect on the subsoil than on the surface soil. Table VI shows results of experiments along these lines. Three different classes of soil were used: 1365 is a virgin clay loam soil; 1366 is the subsoil corresponding to 1365; 1371 is a sandy loam, virgin soil, and 1370 its subsoil; 1374 is a very sandy soil, low in organic matter, and 1373 is its corresponding subsoil. The surface soil was taken as the first eight inches. The subsoil is eight to twenty-four inches.

<sup>24</sup> Bul. 305. U. S. G. S. p. 95.

TABLE VI. EFFECT OF HEAT AND OXIDATION ON THE SOLUBLE PHOSPHORUS IN THE SURFACE AND SUBSOIL

Lab. No.	Kind of soil	Total $P_2O_5$ per cent of soil	N/5HNO <sub>3</sub> soluble $P_2O_5$ per cent of soil	N/5HNO <sub>3</sub> soluble $P_2O_5$ after oxidation, per cent of soil	N/5HNO <sub>3</sub> soluble $P_2O_5$ after oxidation and heat per cent of soil	Organic matter removed, per cent of soil
1365	Surface clay loam.....	.105	.020	.064	.057	2.54
1366	Subsoil clay loam.....	.143	.017	.019	.024	.94
1371	Surface sandy loam.....	.121	.013	.043	.047	1.38
1370	Subsoil sandy loam.....	.093	.008	.007	.019	.08
1374	Surface sand..	.069	.018	.033	.035	.60
1372	Subsoil sand...	.078	.019	.024	.028	.28

The difference in the action of hydrogen peroxide on the phosphoric acid of the soil and subsoil is very marked with the clay and sandy loam soils. In the sand the difference is not so marked, and this is not unexpected for there is not much difference in the amount of organic matter. However, the increase in phosphoric acid in the surface soil is three times that of the subsoil. Heating gives a larger increase with the subsoil than it does with the surface soil after oxidation, though the increase is very much smaller than it is with the surface soil before oxidation. This again points to the conclusion that the phosphoric acid is held by organic matter.

#### CONCLUSIONS

Heating wavellite to 200° for five hours increases the solubility of the phosphorus from 4 per cent to 50 per cent. Heating it to 240° for the same length of time increases the solubility to 100 per cent of the total phosphorus, as determined by fusion with sodium carbonate. Dufrenite when heated to 200° gives but a slight increase in solubility.

Heating a soil to 100° for five hours does not increase the solubility of phosphorus in fifth normal nitric acid. At 130° a small increase takes place and above this temperature the solubility rises rapidly with a rise of temperature, reaching a maximum at about 200°.

By the use of hydrogen peroxide about 90 per cent of the organic matter of the soil can be destroyed.

The solubility of phosphorus is increased on an average about

50 per cent of the total phosphorus in clay and clay loam soils by decomposing the organic matter with hydrogen peroxide. For sandy soils the increase is about 30 per cent of the total phosphorus.

The increase in the solubility of phosphorus obtained by decomposing the organic matter with hydrogen peroxide is always larger than that obtained by heating the soil to 200° to 240°.

After the organic matter has been destroyed by hydrogen peroxide there is no increase in the solubility of phosphorus when the soil is heated to 240°.

The excess of phosphorus obtained from a soil by heating over that obtained from the raw soil is from the same source as that obtained by oxidizing with hydrogen peroxide.

The solubility of the mineral phosphates of the soil does not seem to be increased by heating to 240°.

The early stages of oxidation increase the solubility of the phosphorus more than the later stages. Much the greater part of the increase comes when 25 to 30 per cent of the organic matter has been destroyed. After 60 per cent of the organic matter has been destroyed there is no further increase in the solubility of phosphorus on further oxidation.

The larger increase in the solubility of phosphorus is to be expected in the earlier stages of oxidation for the organic matter becomes soluble without being entirely destroyed, a further decomposition being carried out in the extract by bromine.

The solubility of calcium and manganese is not increased by oxidation with hydrogen peroxide.

The solubility of iron and aluminum in fifth normal nitric acid is increased on oxidation with hydrogen peroxide, the increase following pretty closely the increase in the solubility of the phosphorus.

The increased solubility of phosphorus by oxidation with hydrogen peroxide probably comes, in large part, from precipitated iron and aluminum phosphates, held from solution before the oxidation as part of a complex of insoluble organic matter and compounds of iron and aluminum.

Oxidation increases the solubility of the phosphorus but slightly in subsoils.

Heating after oxidation has a more marked effect on the solubility of the phosphorus in the subsoil than it has in the surface soil.

# Factors Influencing the Availability of Rock Phosphate\*

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E. TRUOG

With the continually increasing use of phosphate fertilizers, the most economical method of using them becomes more and more a matter of importance. Numerous field experiments in this country, conducted at the various experiment stations; and especially at Ohio, indicate quite conclusively that when finely ground raw rock phosphate is used in conjunction with a liberal supply of organic matter, such as farm manure or crop residues, its use is attended with as large or even larger net profit than that attending the use of the more expensive acidulated phosphates. The increased efficiency of raw rock phosphate when supplemented with organic matter has quite generally been explained on the theory that the decaying organic matter exerts a solvent action on the phosphatic material, and thus makes it available to growing crops. This explanation seems reasonable, yet as a matter of fact no direct conclusive experimental evidence has ever been given in its support. Attempts have been made to measure this solvent action by means of laboratory experiments, but as far as the writer is aware, this has never been accomplished satisfactorily. It was with a view of throwing more light on this subject that the present work was undertaken.

## REVIEW OF PREVIOUS WORK ON THIS SUBJECT

One of the earliest experiments in this country is reported by Lupton.<sup>1</sup> Floats were mixed with cottonseed meal and allowed to ferment. Citrate soluble phosphates were determined

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\* The author wishes to express to Prof. A. R. Whitson his appreciation for the criticisms and suggestions given during the progress of the work reported in this bulletin.

<sup>1</sup> Ala. Exp. Sta. Bul. 48, 1893.

from time to time over a period of three months. The results as given are irregular, and though they seem to indicate a slight solvent action, the evidence is far from conclusive.

A later experiment is reported by McDowell,<sup>2</sup> in which floats were thoroughly incorporated with mixed cow and horse manure, placed in a tight barrel and allowed to ferment for a period of about thirteen months. Water soluble, citrate soluble, and insoluble phosphates were determined at the beginning and end of the experiment. The results indicate no increase in available phosphates from the beginning to the end of the experiment.

Holdefleiss<sup>3</sup> composted raw phosphate with various organic materials and inorganic salts. After the organic materials had fermented for eight months, citrate extractions revealed only a very slight solvent action of the composting materials on the raw phosphate.

Pfeiffer and Thurmann<sup>4</sup> made composts of decaying organic materials with raw phosphate and also with superphosphate. After these mixtures had fermented for about six months, analyses showed that the raw phosphate had become but slightly more soluble in citrate extraction, while the solubility of the superphosphate was greatly reduced in citrate extraction, and brought to almost nothing in water extraction. Kröber<sup>5</sup> mixed Thomas slag with sawdust and moist sand. After fermenting for three months, water extractions showed no increase of soluble phosphates.

The experiments cited are open to criticism in that blanks with the organic matter alone were not carried out along with the others. In the course of an experiment of this nature, the available phosphate coming from the organic matter itself may either increase or decrease. This increase or decrease might then be sufficient to entirely mask from chemical measurement any action of the fermenting organic material on the raw phosphates.

Fleischer and Kissling,<sup>6</sup> working with experiments designed to show the effect of moorland soils and peaty substances on insoluble phosphates, found that they rendered appreciable

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<sup>2</sup> Pa. Exp. Sta. Ann. Rpt. (1907-8), 175.

<sup>3</sup> Heiden, Düngerlehre, 2, 509.

<sup>4</sup> Landw. Vers. Stat. (1896), 343.

<sup>5</sup> Jour. Landw. (1909), 57, 32.

<sup>6</sup> Biol. Centr. Agr. Chem. (1883), 155.

amounts of the phosphates soluble in water and ammonium citrate. The solvent action measured here was perhaps due to the acidic properties of the substances used. To a large extent the nature of these substances and their decomposition products are, to be sure, quite different from that of farm manure and crop residues, as used in ordinary farm practice, and hence the results mean little when applied to the problem under discussion.

#### PLAN OF PHOSPHATE EXPERIMENTS

Of the various factors influencing the availability of raw rock phosphate, the present investigation includes a study of the influence of fermenting cow manure and June grass; and the influence of thorough mixing of the rock phosphate with soil.

In the present consideration, availability of rock phosphate is not necessarily taken to mean its solubility in weak solvents only, but also the readiness with which a plant may draw on it for its phosphate supply. To be sure, the more soluble a fertilizer is in weak solvents, the more available it is to growing crops. The converse of this, however, may be far from true. That there is a decided difference between these two conceptions will be clearly shown in the data presented. The investigations here undertaken are all of a laboratory and plant house nature. It is fully recognized that any data obtained in this way can be applied to field conditions only with the greatest caution. However, as already stated, the field experiments relating to the problem under consideration are quite numerous and the data obtained therefrom seem quite conclusive, viz, that organic matter increases the efficiency of floats. To explain why the field results are thus, can only be accomplished in the laboratory and plant house, where outside disturbances may be eliminated and the conditions brought under control.

#### THE INFLUENCE OF FERMENTING MANURE AND GRASS ON THE AVAILABILITY OF FLOATS

##### EXPERIMENT I. COMPOSTS OF ORGANIC MATERIALS AND FLOATS

For the first experiment, jars containing the mixtures indicated, were arranged as follows:

No. 1. 2.7 kg. sand, 25 g. floats, ————

No. 2. 2.7 kg. sand, 25 g. floats, 300 g. grass.



No. 3. 2.7 kg. sand, ————, 300 g. grass.

No. 4. 2.7 kg. sand, 25 g. floats, 300 g. manure.

No. 5. 2.7 kg. sand, ————, 300 g. manure.

*Materials Used* The jars were 1-gallon, glazed, earthenware vessels, each provided with a hole in the bottom. The sand was ground quartzite from Wausau, Wis. analyzing 97.9 per cent silica. The floats consisted of a high grade of finely ground rock phosphate, analyzing 34 per cent phosphoric anhydride. The grass was finely chopped, fresh, green, June grass. The manure was fresh cow manure without litter.

The contents of each jar were thoroughly mixed. The jars were placed in the green house, maintained at optimum water content or nearly so, and stirred occasionally. Active fermentation soon appeared to take place in all the jars containing organic matter.

This work was started about August 1. On the next December 10, the material in each jar was extracted with water, and several weeks later extractions were made with 0.2 per cent citric acid and 1 per cent sodium hydroxide solution. The extractions were made as follows:

*Water Extraction* Water was applied to the jars until the leachings passing through the holes in the bottoms amounted to two liters. These solutions were filtered till clear from all suspended material and then analyzed for phosphoric acid.

*Citric Acid Extraction* Duplicate 1/20 portions, making about 150 g. were weighed out from each jar, placed in flasks and extracted with 300 c. c. of 0.2 per cent citric acid. The flasks were shaken occasionally for several hours and then allowed to stand 24 hours, when 25 c. c. portions were drawn off, filtered and analyzed for phosphoric acid. After standing eight days with frequent shaking, portions were again removed for analysis.

*Sodium Hydroxide Extraction* For this, 1/50 portions were taken from each jar, placed in flasks and extracted with 1 per cent sodium hydroxide solution, according to the method of Stoddart.<sup>7</sup> The resulting solutions were then analyzed, as in the former extractions.

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<sup>7</sup> Wis. Exp. Sta. Res. Bul. 2, 1909, p. 53.

*Method for Determining Phosphoric Acid* Except where otherwise stated the analyses of the various extracts for phosphoric acid reported in this work have been made colorimetrically. The method used and given herewith is the writer's modification of the method given in U. S. Bureau of Soils Bulletin 31, page 45.

A measured quantity of the clear solution to be analyzed is evaporated in a casserole to a volume of about 20 c. c. The solution is made alkaline with sodium hydroxide and just a slight excess added. The casserole is covered and bromine added from a burette through the lip of the casserole in small quantities from time to time, heating on the water bath. After treating in this way for about one hour, dilute nitric acid is added till the bromine is all driven off, and then the solution evaporated to dryness. If this process has been properly carried out, the organic matter will be entirely destroyed and the residue perfectly colorless. The residue is then dehydrated at 110° C. for two hours. To this residue 5 c. c. nitric acid, sp. gr. 1.07, are added with a little water. The solution is filtered, and the casserole and filter washed with water till the filtrate measures about 40 c. c. This leaves most of the silica adhering to the bottom of the casserole, the filter catching any that may wash out. From here on the reagents are used and the color developed as described in the bulletin referred to.

It is essential to use silica-free water and to keep reagents in paraffin lined bottles. In making comparisons the standard and unknown should be at the same temperature, and the dilutions should be such that approximately equal volumes of the standard and unknown are compared.

By this method of treatment with bromine, all organic matter is destroyed and hence cannot interfere with the subsequent development of color. The phosphorus in the soluble organic material is thus determined. This method has been used with success in this laboratory for several years, during which time a large number of determinations have been made. Its value lies in that small amounts of phosphates can be detected and determined without laboriously evaporating down large volumes. Then again, in experiments of this kind, the total volume of solution at hand might not give enough material to determine gravimetrically. On several occasions we have checked the method with the gravimetric method and usually obtained re-

markable concordance. In cases where the quantitative relation between the two varied somewhat, the comparative relation between the members of a set determined colorimetrically still had very closely the same relation as when the set was determined gravimetrically. For rapid comparative work the method is thus reliable and can be depended upon to show small differences which, if of any importance, may then be checked up by gravimetric or volumetric determinations.

Table I gives the results of the analyses of the extracts secured in the manner already described with the different solvents.

TABLE I PARTS OF PHOSPHORIC ANHYDRIDE PER MILLION PARTS OF THE EXTRACTING SOLUTIONS

Jar	Treatment	SOLVENT USED			
		P <sub>2</sub> O <sub>5</sub> by water	P <sub>2</sub> O <sub>5</sub> by 0.2% citric acid 24 hrs.	P <sub>2</sub> O <sub>5</sub> by 0.2% citric acid 8 days	P <sub>2</sub> O <sub>5</sub> by 1% NaOH
1.....	Quartz and floats.....	1.5	116.0	149.0	7.5
2.....	Quartz, floats and grass.....	88.0	68.5	99.0	11.0
3.....	Quartz and grass.....	88.0	26.0	32.0	8.7
4.....	Quartz, floats and manure.....	71.0	88.0	118.0	12.5
5.....	Quartz and manure...	67.0	66.5	57.0	9.3

From this table it is quite clear that neither the fermenting grass nor the manure had any material effect on the solubility of the floats as measured by the extracting water. The contention is not made, however, that there has been no solvent action, but simply that this extraction measures none. The difference between Nos. (4) and (5) indicates nothing since No. (1) must be added to No. (5) to make the two comparable. When this is done, the difference is only about 3.5 per cent, easily within the limit of error of the chemical work.

The extraction with 0.2 per cent citric acid also fails to measure any solvent action that may have taken place. As a matter of fact, when we compare No. (1) with Nos. (2) and (4), it becomes quite clear that the fermenting organic matter has rendered the floats less available, when availability is measured by a solvent such as .2 per cent citric acid. If we consider the 24 hour extraction, and subtract 66.5 from 88, it leaves 21.5 parts. This we may take as the citric acid soluble phosphates coming from the floats in No. (4). When the figure 116 in No. (1) is now

compared with 21.5, it indicates by this method of reasoning that the manure has decreased the solubility of the floats in 0.2 per cent citric acid by more than five times. The same calculation shows that the grass has decreased it about three times. Nevertheless no one would hardly dare to maintain that the mixing of floats with farm manure, as is done in ordinary farm practice, results in the floats becoming less available to the growing crop due to the presence of the manure. This point has already been referred to and will be again taken up in a further discussion. That availability as measured by weak solvents may be entirely different from that as measured by growing crops seems quite evident.

The extraction with sodium hydroxide was made, thinking that perhaps, due to the presence of some iron in the quartz and floats, the phosphates might go over into iron phosphates as soon as made soluble. This being the case, the sodium hydroxide extraction, which readily dissolves iron phosphates, should show that No. (2) is larger than the sum of No. (1) and No. (3) and the same for the other set. The figures do not show this and hence the contention is untenable as far as this method of extraction is concerned.

#### GENERAL INFERENCES FROM EXPERIMENT I

The data in Table I plainly show that the methods used in Experiment I failed to measure any solvent action of the fermenting organic matter on the floats. Why should this be the case? Can it be that there was no action? The fact that the organic matter made the floats less available in 0.2 per cent citric acid solution indicates that the floats were acted upon in some way by the organic matter, either chemically, physically, or both. It seemed quite reasonable to suppose that the organic matter had made the floats soluble to a certain extent, and that this soluble portion was immediately absorbed and held physically and chemically by the organic matter in such a way that the citric acid solution would not extract it. The results of Pfeiffer and Thurmann, as already given, touch directly on this question. Their investigations show that the composting of organic matter with acid phosphate greatly reduces the solubility of the phosphate in water and citrate extractions. These results together with the fact that phosphates more than any other

salts, are known to be absorbed and held by organic matter with such retentiveness that it is difficult to extract them with weak solvents, greatly strengthened the foregoing supposition.

Bacteria and other soil organisms undoubtedly use up a portion of the soluble phosphates in their own life processes. In this case the portion used would be locked up in the bodies of the organisms and would not be measured by a weak solvent. As to the rapidity with which this process takes place the reader is referred to further discussion on page 28.

If the supposition that the soluble phosphates are held physically is correct, then a method which will destroy the remaining organic matter and release the phosphates just before the extraction is made, should prove successful in measuring the solvent action. Also if, as Pfeiffer and Thurmann have shown, the mixing of acid phosphate with organic matter renders the phosphate less easily extractable by a weak solvent, then we will have additional evidence in favor of the contention in the preceding paragraph. For the purpose of testing these possibilities the following experiment was conducted.

#### EXPERIMENT II. COMPOSTS OF MANURE AND FLOATS, AND MANURE AND ACID PHOSPHATE

For the investigations in this experiment the following jars were arranged, observing the utmost care in the selection of materials, the mixing and arrangement of the same and the subsequent care during the period allowed for fermentation.

No. 1.	6 kg. quartz	_____	_____
No. 2.	6 kg. quartz	25 g. floats	_____
No. 3.	6 kg. quartz	_____	100 g. manure.
No. 4.	6 kg. quartz	25 g. floats	100 g. manure.
No. 5.	6 kg. quartz	10 g. acid phosphate	_____
No. 6.	6 kg. quartz	10 g. acid phosphate	100 g. manure

*Materials Used* The jars were 1-gallon glazed earthenware vessels without holes. The quartz was a natural sand from Ottawa, Ill., analyzing 99.13 per cent silica. The floats consisted of a high grade of finely ground rock phosphate analyzing 35.3 per cent phosphoric anhydride. The acid phosphate was acidulated rock, containing 14 per cent available and 15 per cent total phosphoric anhydride. The manure consisted of finely ground air dried cow manure without litter.

The whole series of jars was arranged in duplicate, making twelve in all. The experiment was thus duplicated from the starting of the work with the jars to the end of the chemical work. The phosphatic materials were thoroughly mixed with the quartz before adding the manure. The manure being dry and finely ground was easily, thoroughly, and uniformly mixed. In order to insure uniform bacterial activity, each jar received a little water extract from a rich soil. To each jar 825 c. c. of water were added. The jars were then weighed. This gave a standard weight for each jar at which the per cent of moisture for contents of all was practically the same. The jars were kept in the plant house and watered to standard weight once a week, stirring after each watering.

This method of procedure is preferable, not only in that it makes it possible to keep the material in the different jars at the same moisture content, and hence more nearly under the same conditions, but also in that it eliminates the necessity of making moisture determinations whenever samples are taken for analysis. Whenever samples were taken in the following work, the jars were first watered up to standard weight and the contents thoroughly mixed. A given weight of material from any jar was then strictly comparable to the same weight from any other jar. The amount of material taken from any jar was always recorded in order that the jar might be given the correct new standard weight, at which the moisture per cent of contents was unchanged from former standard weight. This set of jars was started February 26. On the next June 11, samples were taken for the following extractions:

*Water Extractions* Fifty gram samples were put into 600 c. c. Erlenmeyer flasks and 250 c. c. of water added. The flasks were shaken several times and then allowed to stand 24 hours, when portions of the solutions were filtered and analyzed for phosphates.

*Hydrogen Peroxide Oxidation and Subsequent Extraction* In order to investigate the possibility of manure absorbing and holding physically any phosphates that had been made soluble as suggested on page 23, the following method of oxidation and extraction was used. Samples of 25 g. each were put into 300 c. c. Erlenmeyer flasks. From the jars numbered (3) contain-

ing quartz and manure, a double set of samples was taken. To one of these sets now called (3a), .09 g. of floats was added. This is the amount of floats carried by a 25 g. sample from jar (4), which received both manure and floats in the beginning. This set (3a) was taken as a check against the possibility that the oxidation of the organic matter with the hydrogen peroxide might in itself render a part of the floats soluble. A little water was added to each flask and then 3 per cent hydrogen peroxide in portions of 10 c. c. The peroxide was a dilution of Merck's pure 30 per cent solution. The flasks were set on the water bath and gently warmed. After adding about 30 c. c. of the peroxide, practically all of the organic matter was oxidized and the solutions were perfectly colorless. The solutions obtained were neutral to litmus, indicating that the bases and acids liberated, just about neutralized each other. The solutions were filtered and the residue thoroughly washed with water to remove all soluble material. The filtrates were made up to definite volume and portions taken for analyses. The water-insoluble residues from (3) and (3a) were further treated as follows: The filter papers containing part of the material were returned to the respective flasks. These residues were then treated with 0.02 per cent citric acid solution. The extractions covered a period of one hour during which time the flasks were frequently shaken. Filtered portions were then analyzed for phosphates.

Table II gives the results of the analyses of the extracts secured by the methods just described.

TABLE II PARTS OF PHOSPHORIC ANHYDRIDE PER MILLION PARTS OF EXTRACTING SOLUTIONS

Jar	Treatment	BEFORE OXIDATION	AFTER H <sub>2</sub> O <sub>2</sub> OXIDATION	
		P <sub>2</sub> O <sub>5</sub> by water.	P <sub>2</sub> O <sub>5</sub> by water	P <sub>2</sub> O <sub>5</sub> by 0.02% citric acid
1.....	Quartz .....	0.4	0.3	.....
2.....	Quartz and floats.....	1.3	1.2	.....
3.....	Quartz, _____, manure.....	14.0	20.0	.....
4.....	Quartz, floats, manure.....	16.0	20.0	18.6
5.....	Quartz, acid phosphate, _____, .....	9.0	8.5	.....
6.....	Quartz, acid phosphate, manure..	23.0	28.0	.....
3a.....	Same as 3 with floats added just before oxidation.....	.....	20.7	18.2

*Water Extractions before Oxidation* In this table it is to be noticed that the results with water extraction are

similar to those in Table I. When the sum of numbers (2) and (3) is compared with number (4), the difference is again within the limit of error and hence no definite solvent action has been measured. In the case of the acid phosphate, the sum of numbers (3) and (5) just equals number (6). This indicates that soluble phosphates as found in acid phosphate may be mixed and left with decaying manure, in such proportions as used here, for several months, after which water will again readily extract the greater part of the phosphates.

It is important to note, however, that the conditions which influence chemical fixation of phosphates were quite different in the jars containing acid phosphate and manure from those containing floats and manure. The 10 g. of acid phosphate used with the manure were sufficient to maintain the contents of the jars decidedly acid to litmus throughout the experiment. The contents of the jars containing the floats and manure developed a slightly alkaline reaction to litmus, as did also a boiled extract of the same to phenolphthalein. Thus in the case of the acid phosphate, there was little chance for chemical fixation of phosphates, since an excess of acid was always present. In the case of the rock phosphate, it is quite possible that the alkaline manure medium served to fix chemically part of the phosphates that may have been made soluble. It is thus evident that the two cases cannot be compared in every way as was originally planned. However, the results do seem to indicate that the amount of water-extractable phosphates is affected but little by physical fixation under conditions as obtained with the mixtures of acid phosphate and manure used in the present experiment.

The results with acid phosphate may appear to be contradictory to what Pfeiffer and Thurmann found. These investigators, however, used a considerably larger proportion of organic matter to acid phosphate than was used in the present work, which seems to explain the difference; for the larger the proportion of organic material, the more likely is the media to become alkaline and hence bring about chemical fixation and possibly aid physical fixation.

Fixation of phosphates due to bacterial activity would probably also be larger in the alkaline medium than in the acid medium.



*Water Extraction after Oxidation* The figures in the second column show that the extraction with water, after the hydrogen peroxide oxidation, has also failed to measure any solvent action of the manure on the floats.

*Citric Acid Extractions after Oxidation* As was indicated with the acid phosphate, the failure to measure any solvent action, by water extraction does not now seem to be due to physical absorption and retention, but rather to the possibility that the phosphates as soon as made soluble, are again precipitated by the alkaline manure medium or partially used up by bacterial activity. The action of the fermenting manure on the floats would thus result in a splitting up of the particles of floats into very much smaller particles—molecules. If this is the case, then the particles of rock phosphate left after oxidation and water extraction in the residue of No. (4), where manure fermented in the presence of floats, should be more finely divided on the whole than those in No. (3), where the floats were not added until the oxidation process. This being the case, a weak solvent like 0.02 per cent citric acid should extract more phosphates from the former, where the material is more finely divided, and part of which has been freshly precipitated. The figures in the last column of Table II give the results of this extraction. The difference, while being in favor of No. (4), indicating a solvent action of the manure, is easily within the limit of error. The work was repeated, when the results also favored No. (4) but were again within the limit of error.

Had any considerable portion of phosphate become locked up in bacterial cells, then the hydrogen peroxide oxidation would have released this portion again and left it in a condition more soluble in 0.2 per cent citric acid, than the original rock phosphate. The results in the last column of Table II bear directly on this point. While these results do not contradict the possibility that phosphates have been locked up in bacterial cells, yet, they do indicate that under conditions as obtained in this experiment, the locking up of phosphates in this way is a comparatively slow process.

*Citric Acid Extractions Under Varying Conditions* Since the data in Table I show that the manure has made the floats less soluble in 0.2 per cent citric acid solution, it seemed desirable to confirm these data with the present set of jars. It also seemed de-

sirable to investigate how the results might be influenced by varying the length of period of extraction and further by varying the ratio of solvent to weight of material extracted. Accordingly, July 26 the jars were watered to standard weight and three sets of samples taken and extracted according to the following scheme:

Set I. 100 g. sample, with 100 c. c. acid. Ratio, 1:1

Set II. 100 g. sample, with 300 c. c. acid. Ratio, 1:3

Set III. 25g. sample, with 250 c. c. acid. Ratio, 1:10

From jars numbered (3), containing manure and quartz, twice as many samples were taken as from the others. To one-half of these now called (3a) floats were added in an amount equivalent to that carried by samples from jars numbered (4).

The samples were placed in Erlenmeyer flasks, the 0.2 per cent citric acid added and then the flasks were shaken alternately for one-half hour, then let stand one-half hour, when small portions of about 20 c. c. were filtered off and 10 c. c. portions taken for analysis. Any unused solution with filter papers and contents was always returned to the respective flasks. At the end of six and twenty-four hour periods, portions were again removed for analysis, the flasks being shaken occasionally during the meantime. Table III gives the results of these extractions.

TABLE III PARTS OF PHOSPHORIC ANHYDRIDE PER MILLION OF SOLUTION AS EXTRACTED BY 0.2 PER CENT CITRIC ACID UNDER DIFFERENT CONDITIONS OF EXTRACTION.

No.	Treatment of quartz	100 g. sample 100 c. c. acid Ratio, 1:1			100 g. sample 300 c. c. acid Ratio, 1:3			25 g. sample 250 c. c. acid Ratio, 1:10		
		1 hr.	6 hr.	24 hr.	1 hr.	6 hr.	24 hr.	1 hr.	6 hr.	24 hr.
2.....	Floats.....	85	98	122	59	92	116	34	55	86
3.....	Manure.....	122	130	132	46	54	53	18	18	19
4.....	Manure and floats.....	138	144	158	69	96	110	40	59	86
3a.....	(a).....	128	132	148	66	94	106	43	64	94
4a.....	(b).....	16	14	26	23	42	57	22	41	67

(a) Same as (3). with floats added just before ~~oxidation~~ *extraction*

(b) Gives remainders when results under (3) are subtracted from (4) and represents phosphates coming from floats in (4).

It is important to note that in this Table the order of results has been changed considerably by altering the conditions of extraction. Where the ratio of solid to solvent was 1:3 and the

period of extraction 24 hours, which conditions are much similar to those that prevailed in the citric acid extraction reported in Table I, the results are also similar to those reported in that table, viz: that more phosphates were extracted from the floats alone than from the mixture of floats and manure which had been undergoing fermentation in intimate contact for five months. There does not seem to be any good reason why the extractions should not have obtained just as much soluble phosphates from the manure itself in No. (4) as in No. (3). If we assume that the soluble phosphates extracted from the manure in the two cases were equal, then the total extraction of No. (4) as given in the table minus No. (3) gives the quantity of phosphates coming from the floats in No. (4). The figures following (4a) give these quantities after making the subtractions. On comparison we find that these quantities are always much less than those following No. (2). This is again similar to the results in Table I, and indicates that the mixing of floats with manure makes the floats less soluble in weak citric acid solution. It is to be noted, as might be expected, that this difference between Nos. (2) and (4a) becomes less as the ratio of solvent increases.

The figures following (3a) are somewhat irregular, but as a general average they follow No. (4) quite closely. These figures under (3a) will be considered again in a further discussion.

The data in Table III become more easily interpreted when the use of curves is resorted to. The data for Nos. (2), (3), (4), and (4a) have been plotted in Figures 1, 2, and 3, using parts per million as ordinates and time in hours as abscissae.<sup>8</sup>

The curves for No. (3) show that all the soluble material in the manure went into solution during the first one or two hours of extraction, the curves becoming practically horizontal thereafter. The curves for No. (4) rise quickly at first due to the soluble phosphates present in the manure itself. At the end of one or two hours, soluble phosphates ceased to come from the manure and then the rise, being due solely to phosphates coming into solution from the floats, becomes much slower. As a matter of fact, the rise is then slower in all other cases than in

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<sup>8</sup> Drawings for Figures 1, 2, 3, and 4 were made by E. R. Finner of the Dept. of Soils.

No. (2), where no manure was in contact with the floats. Where the ratio was 1:3, the manure has lessened the solubility of the floats so much that No. (2) is ahead of No. (4) at the end of 24 hours, and where the ratio was 1:10 No. (2) has just caught up with No. (4). When the curves for No. (4a) are compared with those for No. (2) the comparisons serve to bring out in a striking way how the presence of the manure in contact with the floats has lessened the solubility of the floats in the citric acid extractions.

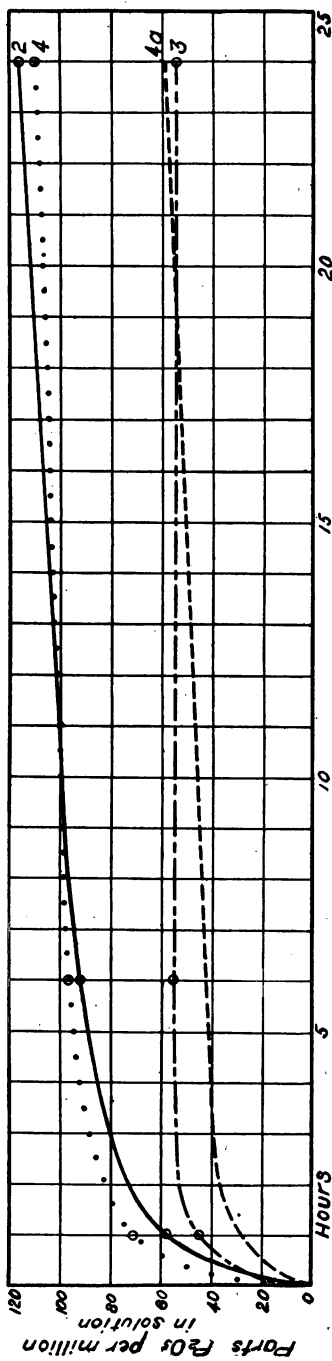
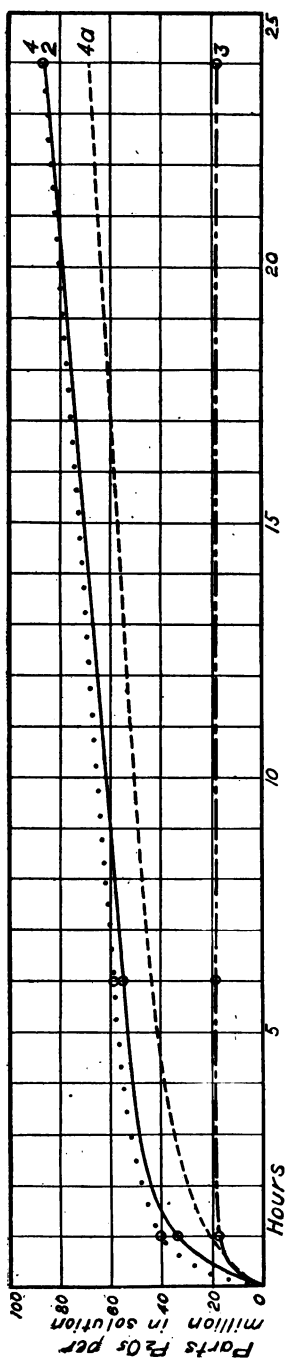
As already stated, the figures under No. (3a) are somewhat irregular and unsatisfactory. If No. (3a) is accepted as a perfect blank on No. (4), then the two extractions where the ratios were 1:1 and 1:3 indicate a slight solvent action of the manure on the floats. The other extraction, however, indicates the opposite. In providing for samples No. (3a) it was necessary to mix the floats with wet manure and quartz. To get thorough and uniform mixing in a case like this is a difficult problem. It seems that the thorough shaking after adding the solvent should have resulted in getting efficient mixing.

In order to check up this part of the work, another set of samples was taken, observing the utmost care to secure efficient mixing. Samples of 100 g. were extracted with 100 c. c. of the acid. In order to secure uniform shaking, the flasks containing the mixtures were shaken in a mechanical shaker for one half hour, after which portions were filtered off and analyzed. The determinations were made volumetrically by solution of the ammonium-phosphomolybdate precipitate in standard alkali and titration of excess with acid. This method was deemed preferable to the gravimetric method, since it is better adapted to show small differences. The results of this work are given in Table IV.

TABLE IV PARTS OF PHOSPHORIC ANHYDRIDE PER MILLION OF SOLUTION, AS EXTRACTED FROM DIFFERENT MIXTURES

No.	Treatment of Quartz	Parts $P_2O_5$ by .2% citric acid
2....	Floats.....	92.0
3....	Manure.....	169.0
4....	Manure and floats.....	193.5
3a....	(a).....	193.0

(a) Same as No. 3 except that floats were added just before extraction in an amount equal to that carried by No. 4.



### Legend for Figures 1, 2 and 3.

- ..... No. 4 - Quartz, manure and floats.
- No. 3 - Quartz, manure and floats.
- No. 2 - Quartz and floats.
- No. 4a - No. 4 minus No. 3.

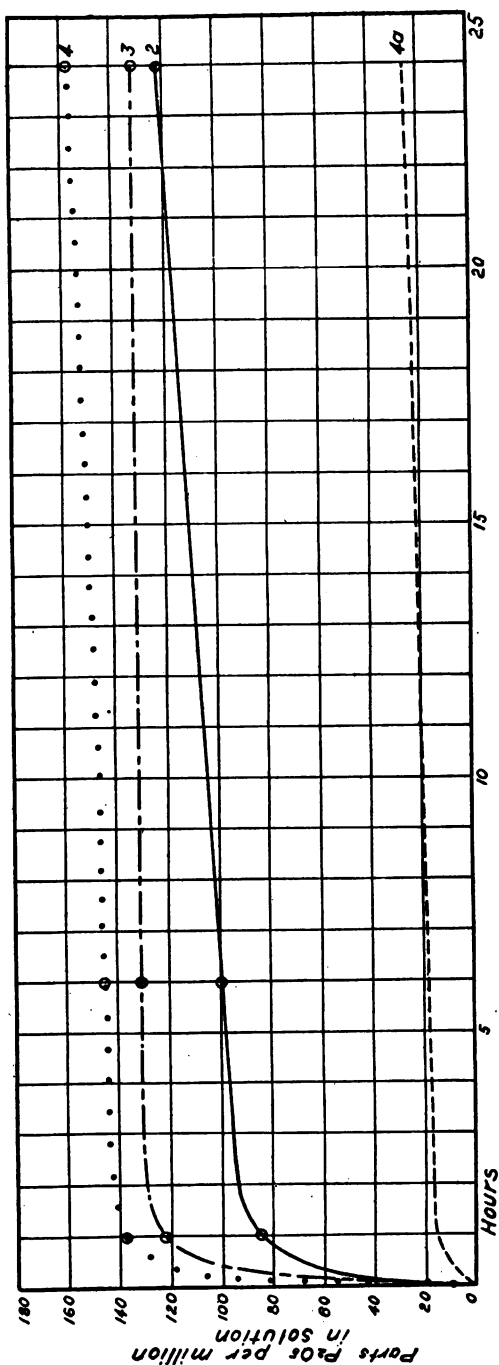


Figure 3. Rate of solution of phosphates, similarly as in Figure 1, when the ratio of solid to solvent was 1:1

From this table it is evident that little solvent action has been measured, since No. (4) and No. (3a) are practically the same. The other results are exactly in the same order as in Table III. The vigorous shaking has resulted in bringing much more phosphates into solution in a short time than was obtained by the previous extractions.

#### GENERAL CONSIDERATION OF EXPERIMENTS I AND II

From the data which have been presented so far it is quite evident that the solution of the problem under consideration is attended with many difficulties. Why the presence of the manure in contact with the floats should so greatly reduce the solubility of the floats in dilute citric acid solution is not altogether clear. That it is not due to the locking up of the phosphates in bacterial cells is evident from the fact that the lessened solubility is the same whether the extraction is made immediately after mixing the floats with the manure or after allowing the floats-manure mixture to ferment for several months. It might be suspected that the alkalinity of the manure could have been sufficient to neutralize enough of the acid used in the extraction to account for the weakened action of the floats, in the floats-manure mixture. In order to test this point, portions of the same extracts as used for the analyses reported in Table IV, were titrated with standard alkali. The citric acid solution used to extract the mixture of floats and quartz had retained 83 per cent of its original strength; that used to extract the mixture of floats, manure and quartz had retained 75 per cent of its original strength. In these cases only 100 c. c. of acid had been used to extract 100 g. of material. The weakening of the acid due to the alkalinity of the manure thus seems entirely too small to account for a lowering of the solvent action by several times.

That the particles of floats adhere to and are held by the fragments of manure seems very probable, for on taking samples of the mixtures and shaking them with water in a flask, and then letting stand, no separation of floats is seen to take place. Just as soon as the organic matter is partly oxidized with hydrogen peroxide, the floats settle out and can be readily seen on the bottom of the flask. It seems quite possible that the moist manure covers the particles of floats with slimy films,

which the dilute citric acid penetrates slowly. It also seems probable that the floats made soluble by the fermenting manure, may be precipitated by the alkaline medium, resulting in the formation of fine particles in contact with the manure. Of all the particles of floats in the mixture, these fine ones precipitated in intimate contact with the manure would probably be the most efficiently protected from a weak solvent. If this has been the case, then it seems that the oxidation method, as performed, should prove successful. The difficulty, however, is that the extraction dissolves so large a quantity of phosphates from the floats which have not been acted upon by the manure, as to entirely mask from definite chemical measurement any solvent action that may have taken place. This becomes more apparent when we consider the fact that in the analyses of the citric acid extractions made after previous oxidation, as reported in Table II, the amount of solution used for analyses represented the action of 0.2 g. of manure on 0.05 g. floats. Perhaps after a much longer period of fermentation it will be possible to demonstrate a definite solvent action by some one of the methods already described. While the results thus far reported generally favor a slight solvent action, the amounts are far too small on which to base definite conclusions.

#### SOLVENTS PRODUCED BY FERMENTING MANURE

The statement is frequently made that rotting manure develops various organic acids that help to bring plant food material into solution. The fact that the fermenting manure and June grass used in the foregoing work became alkaline after four to five months of fermentation, would seem to indicate that sufficient bases are present during the early stages of decomposition to neutralize any free acids other than carbon dioxide that might be formed. When manure is applied to cultivated soils, under conditions as obtained in ordinary farm practice, it disappears quite rapidly, indicating very little formation of the more or less inert humic acids and humus compounds. However, as the following references indicate, the addition of manure to a soil greatly increases the carbon dioxide production of that soil.

Boussingault and Lewy<sup>9</sup> have shown that the air of rich soils

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<sup>9</sup> Johnson, *How Crops Feed*, p. 219.



containing considerable organic matter, may contain many times as much carbon dioxide as the air of soils poor in organic matter.

Working with a soil which had not received manure for some time, Stoklasa<sup>10</sup> was able to show that the addition of manure in the proportion of 10 g. manure to 1000 g. soil, which is an application of about 12 tons per acre, calculating two and one half million pounds per acre eight inches, more than doubled the carbon dioxide production of the soil during the following 32 day period. With a soil that has been manured more frequently, the same addition of manure still increased the carbon dioxide production appreciably.

Sewerin<sup>11</sup> has shown that an unsterilized soil may produce ten to twenty times as much carbon dioxide as a sterilized soil.

The application of manure to a soil serves not only to inoculate that soil with much active bacterial life, but also to furnish food and improve the conditions for that growth. The sum total of this effect greatly increases the carbon dioxide production of the soil, since it has its origin in the respiration of the soil life and the oxidation of the organic matter.

It seems reasonable to believe that the solvent action of manure when applied to normal soils is largely due to the increased carbon dioxide production. In the mixtures of organic matter used in the foregoing work, it appears that carbon dioxide was the only free acid formed and hence would be the only acidic substance that could have exerted a solvent action on the floats. It is interesting to note that the solvent action ascribed to bacterial activity is often much greater when carbohydrates are present furnishing proper material for the production of carbon dioxide.<sup>12</sup> Since the addition of manure favors bacterial activity it is probably also accompanied by increased action on insoluble phosphates due to the enzymes produced by the bacteria.<sup>13</sup> Bacteria, of course, use up soluble phosphates in their own metabolism, and thus their growth may be followed by a decrease in soluble phosphates for the time being, as Sewerin<sup>14</sup> has shown. We must, however, not fail to recognize that the

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<sup>10</sup> Centbl. Bakt., 2 Abt., 1911, **29**, p. 408.

<sup>11</sup> Centbl. Bakt., 2 Abt., 1910, **28**, p. 561.

<sup>12</sup> Mich. Exp. Sta. Spec. Bul. 43, 1908, pp. 28-29.

<sup>13</sup> Stoklasa. Centbl. Bakt., 2 Abt. 1911, **29**, p. 499.

<sup>14</sup> Centbl. Bakt., 2 Abt., 1910, **28**, p. 561.

most productive soil conditions are generally accompanied by intensive bacterial activity, indicating that the two go hand in hand.

Hopkins<sup>15</sup> suggests that the nitric acid formed in the process of nitrification may act on insoluble phosphates and make the material available to growing crops. Where the fermenting material becomes alkaline as was the case in the present work, the nitric acid would be neutralized as soon as formed and hence could not act on the floats. A determination of nitrates made on the fermenting manure mixtures used in the foregoing investigations gave 141 parts of nitrates per million of the dry material, showing that even though active nitrification was taking place, the nitric acid formed was insufficient to neutralize all the alkalinity developed at the same time.

### EXPERIMENT III EFFECT OF MANURE ON THE CARBON DIOXIDE PRODUCTION OF SOILS

In order to determine how the manure used in Experiment II might affect the carbon dioxide production of a soil, the following experiment was undertaken: Four 2-gallon glazed earthenware jars were used. Each was provided with a bent glass tube that passed through the center of the bottom of the jar. The connections of the tubes with the jars were made by means of rubber stoppers and sealing wax. A small perforated tin cover was placed over the opening of each tube inside the jars. One kilo of gravel consisting of particles about the size of small peas was placed in each jar, just covering the perforated covers. On top of this gravel 7 kg of soil were placed. To the soil used in two of the jars, 200 g. of manure were previously added and mixed. The soil used was sandy and low in organic matter. The manure used was air-dried cow manure, similar to that used in experiment II.

To each jar 800 c. c. of water were added and the jars weighed. The jars were placed in the plant house and contents stirred and watered occasionally. After several weeks, active fermentation was taking place where manure had been added.

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<sup>15</sup> Soil Fertility and Permanent Agriculture, p. 197.

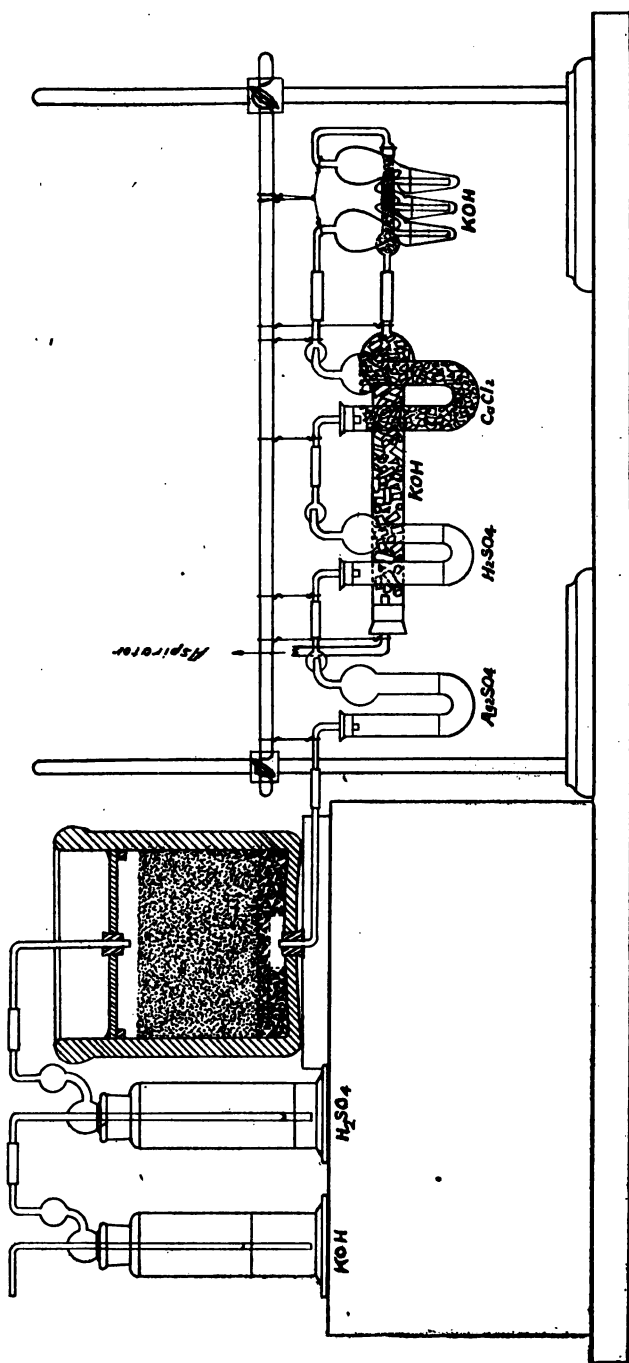


Figure 4. Apparatus for determining the carbon dioxide production of soils

The jars were watered to their original weight. A glass cover provided with a hole was fitted into the top of each jar. The cover was held in place about three inches from the surface of the soil by means of a ring support passing around the inside edge of the jar resting on supports passing to the bottom of the jar. The cover was sealed to the jar with putty and the jars covered with a coat of varnish, making the whole air tight. The hole in the cover was provided with a rubber stopper carrying a bent glass tube. This tube was connected to a guard bottle containing sulphuric acid, and this to another containing caustic potash. A train of apparatus as ordinarily used in the determination of carbon dioxide was attached to the tube leading from the bottom of the jar. Figure 4 shows the entire arrangement of apparatus used.

After testing for leaks, a stream of air was drawn through until the apparatus was practically freed of carbon dioxide. A 20-liter aspirator was used. After standing 12 hours a slow stream of air was drawn through with Geissler bulb in place, till the carbon dioxide was practically all drawn out of the jar. The increase in weight of the Geissler bulb thus gave the weight of carbon dioxide formed in the jar from the time it had been freed of that gas. It is important to note that there was sufficient air in the jars to amply supply the oxygen needed during a twelve-hour period, when no air was drawn through. In the pore space of the soil and above it to the cover there were four to five liters of air, containing sufficient oxygen for the production of several times as much carbon dioxide as formed. The temperature during the determination was that of the laboratory, about 20° C. The results of this work are given in Table V calculated to twenty-four-hour periods.

TABLE V PRODUCTION OF CARBON DIOXIDE IN MANURED AND UNMANURED SOILS

Grams of Carbon Dioxide Formed in 24 Hours

Trial	Unmanured 7 kg. soil			Manured 7 kg. soil, 200 g. manure			Increase due to manure
	Jar 1	Jar 2	Av.	Jar 3	Jar 4	Av.	
1.....	.1093	.1590	.1341	.8559	.7567	.8063	.6722
2.....	.0884	.1530	.1197	.7219	.7811	.7515	.6318

The data in this table show that the manured soil produced about six times as much carbon dioxide as the unmanured soil. The 200 g. of air dried manure used, represented about 800 g. of fresh cow manure. This 800 g. of manure to 7 kg. of soil represents an application of about 150 tons per acre if we assume that the manure is mixed with the surface eight inches, and that this eight inch layer over an acre weighs 2,500,000 pounds. Calculating from these data, an application of twenty-five tons of manure per acre would suffice to just double the carbon dioxide production of the surface eight inch layer of a soil such as used. One kilo of the unmanured soil produced carbon dioxide at the rate of about 18 mg. in 24 hours, and calculating from the increase due to the manure, 10 g. of the wet manure produced 8 mg. of carbon dioxide in 24 hours.

Stoklasa<sup>16</sup> working with a soil low in organic matter, found that 1 kg. produced 14 mg. in twenty-four hours. By adding 10 g. of fresh cow manure to 1 kg. of soil, the carbon dioxide production was just about doubled. This represents an application of about 12 tons per acre, calculating on the same basis as before.

Considering that the two experiments were carried out with different manures and soils in different proportions and by methods considerably different, it seems remarkable that the results should concord so well. Had a smaller proportion of manure to soil been used in the present work, as Stoklasa did, the amount of carbon dioxide arising, due to the addition of 10 g. of manure would undoubtedly have been larger and more nearly equal to Stoklasa's figure.

#### EXPERIMENT IV. SOLVENT ACTION ON FLOATS OF SOIL AIR FROM MANURED AND UNMANURED SOILS

In order to demonstrate that the carbon dioxide production of the manured soil had been increased sufficiently through the addition of the manure to bring about a measurable solvent action on floats, the following experiment was performed with the set of jars used in Experiment III.

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<sup>16</sup> *Centbl. Bakt.*, 2 Abt., 1911, **29**, p. 408.

The apparatus with jar connected having been freed of carbon dioxide twelve hours since, a battery of four Erlenmeyer flasks was attached in position in place of the Geissler bulb. Each flask contained one-half gram of floats and 200 c. c. of water freed of ammonia and carbon dioxide. Forty liters of air were then drawn through by means of an aspirator, the arrangement being such that the passing air bubbled through the liquid in the battery of flasks. The solutions in the four flasks were poured together, filtered and analyzed for phosphates. The battery of flasks, containing a fresh supply of floats and water was again placed in position as before and two liters of air drawn through twice per day during four successive days. The solutions were filtered and analyzed as before. Table VI gives the results of this work.

TABLE VI PHOSPHATES DISSOLVED BY CARBON DIOXIDE FROM MANURED AND UNMANURED SOIL

Air from Soil	Mg. $P_2O_5$ dissolved by	
	(a) One aspiration of 40 liters.	(b) 8 successive aspir- ations of 2 liters each.
Unmanured.....	0.52	1.08
Manured.....	1.04	2.20

These data show conclusively that the addition of manure to a soil may increase the carbon dioxide production of that soil to such an extent that an increased solvent power of the soil air on floats is readily demonstrated. The addition of the manure has just about doubled the solvent power under the conditions as obtained.

By this method it is possible to show that fermenting manure during a period of a few hours, gives rise to sufficient carbon dioxide to measurably exert a solvent action on floats. With the methods used in the previous work, little or no action could be measured after four or five months. This apparent inconsistency will be taken up in the final discussion.

## AVAILABILITY OF FLOATS AS INFLUENCED BY THOROUGHNESS OF MIXING

### EXPERIMENT V. INFLUENCE OF THOROUGHNESS OF MIXING

For the purpose of determining what effect thoroughness of mixing of the floats with the soil medium might have on the availability of the phosphates to a growing crop, the following experiment was carried out. A set of four-gallon glazed earthenware jars were filled with 50 pounds of quartz and treated as follows:

Set No.	Treatment
2.....	Blank.
4.....	15 grams rock phosphate ordinarily mixed.
6.....	15 grams rock phosphate thoroughly mixed.
8.....	7½ grams rock phosphate ordinarily mixed.
10.....	7½ grams rock phosphate thoroughly mixed.
12.....	30 grams acid phosphate.
14.....	15 grams acid phosphate.
16.....	7½ grams acid phosphate.
18.....	3½ grams acid phosphate.

This set was arranged in duplicate. The quartz, floats, and acid phosphate were similar to the materials used in Experiment II. In order to secure what is called thorough mixing of the floats with the quartz, the floats were first carefully mixed with 1 kg. of quartz and then this mixture with the remainder of the 50 pounds of quartz. In the case of what is called ordinary mixing, the floats were sprinkled on the surface of the dry quartz and then mixed with a suitable instrument. The acid phosphate was similarly applied.

On March 7 the jars were planted to corn. Distilled water was used throughout the experiment. After the corn was up and growing well, it was thinned to three plants per jar. A nutrient solution containing the essential elements except phosphorus was applied once a week. It was deemed more preferable to apply this frequently in small quantities diluted with a large amount of water, than all at once in the beginning, since a large quantity of salts in solution may of itself exert a considerable solvent action on floats. The jars were kept in the green house and contents watered and stirred when needed.

Up to April 1, when the corn had reached a height of about 15 inches, the corn in all the jars except the blank and that

receiving 30 g. acid phosphate was of about equal appearance and growing well. Where 30 g. of acid phosphate were used, the excess of acidity seemed to be injurious. After the fourth week the corn in the jars where the floats had been thoroughly

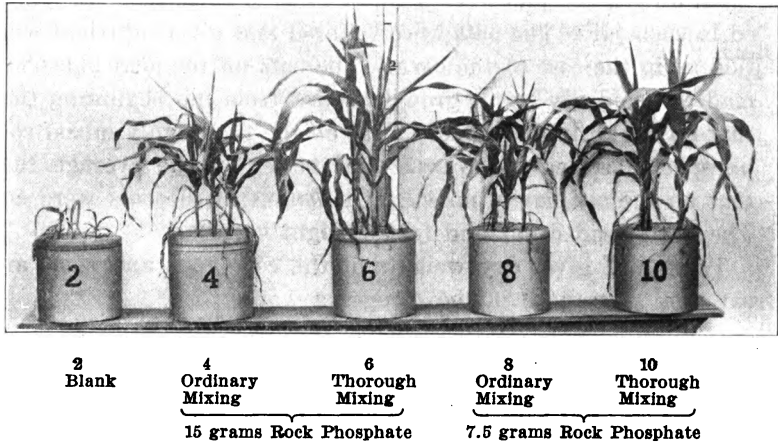


Figure 5. The effect on the growth of corn of thoroughness of mixing rock phosphate with the soil medium.

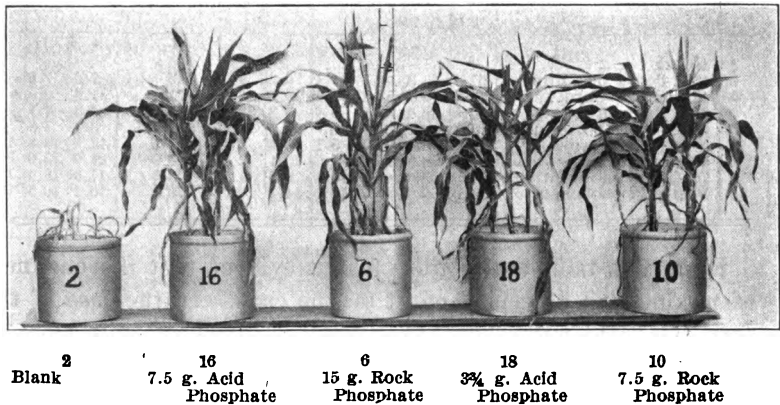


Figure 6.—The effect of acid phosphate on corn compared with the effect of thoroughly mixed rock phosphate.

mixed, was beginning to show a better growth than that where the floats were ordinarily mixed. That on the acid phosphate, except where 30 g. were used, was beginning to show a slight advantage over all the rest. Pictures were taken April 29, just as the corn was beginning to tassel out. (See Figures 5 and 6).



On May 22 the crop was harvested. Some of the corn had formed small nubbins.

The roots were removed and separated from the quartz with a sieve, returning the quartz to the respective jars.

On July 2 the jars were planted to oats, 20 seeds being planted in each jar. The oats were watered and given nutrient solution as in the case of the corn. The oats on the acid phosphate made a decidedly better growth almost from the beginning than that on the rock phosphate. That on the thoroughly mixed rock phosphate seemed to be somewhat better on the average than that on the ordinarily mixed. On August 3 the oats were cut. They had headed out and formed light kernels.

Table VII gives dry weights of the corn tops and roots and oat tops as secured in the foregoing work.

TABLE VII EFFECT OF DIFFERENT PHOSPHATE TREATMENTS ON THE YIELD OF CORN AND OATS

Set No.	Treatment	Corn						Oats		
		Tops			Roots			Tops		
		a	b	av.	a	b	av.	a	b	av.
2	Blank.....						0.8			2.0
4	15 g. floats ord. mix....	51.8	35.8	43.8	10.1	6.6	8.3	52.1	7.0	7.2
6	15 g. floats thoro. mix....	57.8	59.0	58.4	14.7	10.6	12.6	71.0	11.8	6.5
8	7½ g. floats ord. mix....	44.5	37.4	40.9	8.4	8.8	8.6	49.5	4.3	4.7
10	7½ g. floats thoro. mix....	57.8	56.7	57.2	13.1	10.7	11.9	69.1	6.4	7.2
12	30 g. acid phosphate.....	24.8	19.4	22.1	5.7	3.4	4.5	26.6	12.5	13.8
14	15 g. acid phosphate.....	73.0	58.5	65.7	11.5	13.1	12.3	78.0	24.0	19.0
16	7½ g. acid phosphate.....	71.9	63.0	67.4	12.4	9.9	11.1	78.5	17.0	18.4
18	3½ g. acid phosphate.....	68.0	66.5	67.2	10.4	14.1	12.2	79.4	16.2	16.5

From this table and Figure 5 it is evident that thoroughness of mixing had a decided effect on the crop. In the case of the corn the yield with thorough mixing approaches quite close to the yield with acid phosphate. When 30 g. of acid phosphate were used, the excess of acidity greatly reduced the yield. Although the quartz was thoroughly sifted and mixed in order to remove the corn roots, yet on the average the effect of the thorough initial mixing is still apparent on the oat crop. The yield of oats with floats is far behind the yield with acid phosphate, indicating that oats have a weaker feeding power for rock phosphates than corn. However, the cases are not strictly comparable, since the corn growing first had a chance to use up the more easily soluble phosphates in the floats.

Since the jars contained no organic matter or carbohydrates of any kind, it does not seem probable that the floats were made soluble through bacterial activity. Had the floats been made soluble by any other means than the plants themselves, then this soluble portion would have become distributed throughout the contents of the jars, and then there should not be this large difference between ordinary and thorough mixing. It seems more reasonable to believe that the plants themselves exert a marked solvent action on floats. Since all attempts to find appreciable amounts of acid root excretions other than carbon dioxide have failed, this solvent action must be attributed to the carbon dioxide given off by the roots.

#### IMPORTANCE OF THE CARBON DIOXIDE PRODUCTION OF PLANT ROOTS

Stoklasa<sup>17</sup> has measured the carbon dioxide production of roots of various plants. Calculated to dry weight, he found that 100 g. of twenty-five-day-old wheat roots produced 2.54 g. of carbon dioxide in 24 hours. Similarly, fifty-day-old clover roots produced 5.8 g. of carbon dioxide. From his data obtained during the whole vegetative period, he calculated that the average carbon dioxide production of wheat roots per day for the vegetative period is 60 kg. per ha.

Kossowitsch<sup>18</sup> calculated from his data that the roots of one ha. of mustard plants produce during the vegetative period 2250 kg. of carbon dioxide. Assuming the following reaction:  $\text{Ca}_3(\text{PO}_4)_2$  plus  $4\text{CO}_2$  plus  $4\text{H}_2\text{O}$  equals  $2\text{Ca}(\text{HCO}_3)_2$  plus  $\text{CaH}_4(\text{PO}_4)_2$ , he calculates from the analyses of the mustard plants that twenty times as much carbon dioxide is produced as is required to bring the phosphates used by the plants into solution.

Theoretically, according to the reaction above, 1.24 parts of carbon dioxide dissolve one part of phosphoric anhydride.

Kröber,<sup>19</sup> calculating from the phosphates brought into solution by the carbon dioxide formed by cultures of yeast, found that it required sixty-eight to sixty-nine parts of carbon dioxide to bring one part of phosphoric anhydride into solution. From

<sup>17</sup> Centbl. Bakt. 2 Abt., 1905, 14, p. 735.

<sup>18</sup> Russ. Jour. Exp. Landw. 1904, 4, p. 493.

<sup>19</sup> Jour. Landw., 1909, 57, p. 38.

this he concluded that it takes much larger quantities than Kosowitsch considered necessary.

Perhaps the reason Kröber finds that such large amounts of carbon dioxide are necessary, is due to the conditions that prevailed in his experiment. The volume of his solution, it appears, amounted to 250 c. c. and 22.75 g. of carbon dioxide were developed during the fermentation. A liter of water will dissolve only about two grams of carbon dioxide under ordinary conditions. As long as the water is saturated any additional carbon dioxide passes off and does not affect the amount of a substance that will be brought into solution. Had his volume of solution been larger, the efficiency of the carbon dioxide undoubtedly would have been increased.

Calculating from the average results of various investigators<sup>20</sup> it is found that in carbon dioxide saturated water, at ordinary conditions of temperature and pressure, it takes about six parts of carbon dioxide to dissolve one part of phosphoric anhydride from precipitated tri-calcium phosphate. To dissolve the same amount of phosphoric anhydride from rock phosphate takes three to four times as much carbon dioxide. The efficiency of the carbon dioxide in bringing phosphates into solution depends entirely on the conditions under which the carbon dioxide acts and what the nature of the insoluble phosphates are.

It is most important to recognize that the carbon dioxide given off by the plant roots exercises its solvent action under conditions which have never been imitated in the laboratory. The reaction proceeding when carbon dioxide acts on phosphates must be considered as of the nature of a balanced action. The plant immediately absorbing the portion made soluble, the reverse reaction is prevented and hence the carbon dioxide works under a maximum efficiency in bringing phosphates into solution. How efficient a solvent carbon dioxide is for phosphates under these plant root conditions is still a matter of mere conjecture. Perhaps the best guess that can be made is that the efficiency lies somewhere between the theoretical and that which is obtained by the methods now used in the laboratory.

From the foregoing considerations, it seems probable that because of the carbon dioxide given off by the roots, plants are able to secure a large portion of their phosphate supply from inso-

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<sup>20</sup> Comey, Dict. Chem. Sol's. p. 298.

luble sources, provided the insoluble sources are well distributed and thus bring particles in near contact to the whole root system of the growing plant. It is now becoming quite generally believed that the efficiency of acid phosphate is due not to the fact that the phosphate remains more soluble, but to the fact that the phosphate becomes distributed quite thoroughly through the feeding area of the soil. This seems all the more probable from the general recognition that soluble phosphates are usually soon precipitated from solution when applied to a soil.

#### GENERAL DISCUSSION

It seems reasonable to believe that in most cases any factor which will aid in giving better distribution of the rock phosphate through the soil or aid in bringing the phosphate in more general contact with the plant roots, will result in making that phosphate more available to the growing crop. If this conception of availability is accepted, then it is apparent that for the subject under consideration, availability as measured by a weak solvent may be entirely different from availability as measured by a growing crop. Moreover, it is to be recalled that the mere mixing of floats with manure resulted in the floats becoming less available when availability is measured by the 0.2 per cent citric acid solution. Yet, the numerous field experiments point quite conclusively to the belief that this practice makes the rock phosphate more available to growing crops.

Since it is easy to demonstrate that the addition of manure to a soil results in a large increase of the carbon dioxide production of that soil, and this increase in carbon dioxide production can be shown to result in an increased solvent action on floats, it seems reasonable to conclude that fermenting manure does exert a solvent action on floats which are in contact with the manure. Since the laboratory composting experiments fail to measure this solvent action satisfactorily, it only remains to be said that the methods so far devised for these experiments are not suited for the purpose at hand.

We must not fail to recognize that the conditions in the laboratory composting experiments are far different from the conditions obtained in the field, where mixed manure and floats are applied. In the field the movements of soil water and the presence of nearby growing plant roots are constantly at work re-

moving from the particles of manure in contact with floats, the phosphates made soluble. Under such conditions, as already explained, a solvent action may progress under maximum efficiency. The phosphates made soluble, if not used immediately by a growing crop, are distributed by the movements of the soil water and precipitated in fine particles over a large area in fit condition to be used by succeeding crops.

With the composts used in the laboratory experiments the soluble portion is not removed from the sphere of action and hence the solvent power may be checked as soon as the particular solvent becomes saturated. The efficiency of the carbon dioxide formed by the fermenting manure in bringing phosphates into solution depends upon the rate at which the already soluble portion is removed, and upon the amount of water present. When the water present becomes saturated with carbon dioxide, and the phosphates already in solution are not removed, any further production of carbon dioxide is dissipated to the atmosphere and does not aid in bringing phosphates into solution. Accepting this conception of the conditions that prevail in the laboratory composting experiments, then these experiments should reveal a slight solvent action, and this is just what they do on a general average.

It seems that the mixing of floats with manure should result in favorable conditions for the influence of bacterial activity on the floats. Each particle of manure in the soil undoubtedly becomes a center of increased active bacterial life, and if floats are in contact with the manure, the chances for bacterial action on the floats are greatly increased. Although the bacteria may use up the phosphates in the floats in their own metabolism, yet ultimately after death and decay of these bacterial cells, the phosphates contained therein will have become more finely divided and better distributed, resulting in an increased availability to succeeding crops.

Undoubtedly the practice of thorough mixing of the floats with manure results in a better initial mechanical distribution of the floats with the soil than is generally obtained, when the floats are applied directly to a soil. To thoroughly mix a pound of floats with several tons of soil would be a difficult matter if the floats were added directly to all of the soil. If the floats were first mixed with a hundred pounds of the soil and then this with the remaining soil, much more efficient admixture would be

possible. To a certain extent, this same factor influencing thoroughness of mixing is secured when floats are first mixed with manure, previous to application. The direct application of floats to a tight clay soil low in organic matter may result in the floats becoming locked up in local areas which represent a comparatively small portion of the cultivated layer. Further distribution either physically or chemically would be a slow process in such instances. The use of manure and other organic materials prevents such cases as this.

It is a matter of common occurrence to find fragments of manure or other organic materials present in the soil completely entangled with plant roots.<sup>21</sup> The roots are attracted chemotactically by the soluble plant food in the manure. When floats are mixed with manure, this same action may attract the roots of the growing crop to the centers of rock phosphate supply and thus to a certain extent the phosphate is made more available to the crop because of the manure.

The use of manure and crop residues favors the activity of many of the very same agencies that unlock the insoluble phosphates of the granites and make them available to growing crops. Can there then be any question but that these same organic substances help to make floats available to growing crops!

#### SUMMARY

The laboratory experiments in which organic matter is composted with raw phosphates reveal on a general average only a slight solvent action of the fermenting material on the phosphates. The amount of solvent action is always within the limit of experimental error. Considering the conditions that prevail in these composting experiments, this result is exactly in accord with what is to be expected, for, since carbon dioxide is the only free acid formed in these composts, only a slight solvent action should be measured. The amount of the solvent action measured is limited to the amount of phosphate which the carbon dioxide-charged water can hold in solution.

In the composting experiments, the dissolved phosphates and carbonates are not removed from the field of action, and hence the reaction bringing phosphates into solution quickly reaches a state of equilibrium, after which any further production of car-

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<sup>21</sup> Hall—Fertilizers and Manures, p. 290.

bon dioxide is dissipated to the atmosphere and aids nothing in bringing phosphates into solution.

Under field conditions the movements of soil water and the feeding of crops are constantly removing dissolved phosphates and carbonates from the little centers of solution, existing as fragments of organic material where intensive carbon dioxide production takes place. This continual removal of the dissolved substances results in conditions under which the efficiency of carbon dioxide as a solvent is greatly increased.

*Since in the composting experiments the dissolved substances are not removed as under field conditions, it must be concluded that the laboratory experiments fail to imitate field conditions with regard to a most vital consideration.*

The mere mixing of floats with manure makes the floats less soluble in 0.2 per cent citric acid solution. This lessening in solubility takes place immediately on mixing the floats with the manure. From this it must be concluded that the availability of phosphates as measured by a solvent like 0.2 per cent citric acid may be entirely different from availability as measured by a growing crop.

The availability of raw phosphates as measured by a growing crop is influenced not only by its solubility in weak solvents, but also to a large extent by the thoroughness with which it is distributed through the feeding area of the soil.

When floats are thoroughly mixed through the feeding area of the soil, it appears that some species of plants are able to secure nearly an adequate supply of phosphates from the insoluble floats. It seems that the carbon dioxide given off by the plant roots is instrumental in bringing the phosphates into solution and thus making the floats available.

*The addition of manure to a soil greatly increases the carbon dioxide production of that soil. It is easy to demonstrate that this increased carbon dioxide production exerts during a short period a measurably increased solvent action on floats. When floats are mixed with manure, the raw phosphate is placed at the centers of carbon dioxide production and bacterial activity, giving ideal conditions for solution and distribution of the phosphates.*

It seems that the addition of floats to a tight clay soil low in organic matter may result in the floats becoming locked up in

local areas from which further mechanical and chemical distribution of the phosphates would be very slow.

After carefully considering the more important factors affecting the availability of floats to growing crops, there seems to be little question but that the use of organic matter in connection with the floats increases this availability. The organic matter brings about this increased availability by favoring a more efficient initial mechanical distribution of the floats with the soil and by favoring the chemical and biological processes that give rise to carbon dioxide and other agencies which attack floats and ultimately give the material a finer and more uniform distribution through the soil.





# Studies of the Nutrition of the Pig

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## NOTES ON THE CREATININ EXCRETION OF THE PIG

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E. V. McCOLLUM.

With the discovery by Folin that the amount of creatinin excreted by an individual is nearly constant, together with his contribution of a quantitative method for its determination, which in accuracy and ease of operation leave little to be desired, we are in possession of a valuable aid to the study of certain phases of protein metabolism. The work of Folin<sup>16</sup> leaves little doubt that there are normally two kinds of protein metabolism going on simultaneously in the animal body. One is the essential or endogenous and is practically constant and represents the processes of cellular activity. The other is the exogenous which is exceedingly variable and represents the prompt conversion of the nitrogen of the food protein into the end products of metabolism, principally urea. The magnitude of this type depends upon the protein intake and at moderately high levels is proportional to it. The endogenous type results in the formation of a distinct group of end products, the only one of which we have any definite knowledge being creatinin.

Folin, working with men, did not attempt to eliminate entirely the exogenous type of metabolism by long continued feeding of a ration very low in nitrogen. After ten days on diets containing less than a gram of nitrogen a day the men in his experiments still eliminated more than 60 per cent of the total nitrogen of the urine as urea. Since urea is the nitrogenous constituent suffering the most marked diminution in quantity as the exogenous metabolism is diminished, it did not appear from his experiments how nearly the latter

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<sup>16</sup> Folin, *Amer. Jour. Physiol.*, **13**, 84 (1905).

type had been eliminated. In order to arrive at a condition in which the whole of the protein metabolism is of the endogenous type, provided this is possible, it would be necessary to keep an animal during a long period on a diet free from nitrogen, but supplying all the other necessary elements and organic complexes necessary to normal metabolism. Doubtless, also, these things should be supplied in generous but not excessive amounts, and the animal should be kept under conditions as nearly normal as possible. These conditions are difficult to realize with any animals ordinarily employed in metabolism studies. A considerable experience with the pig as a subject in our work with the mineral elements led me to the belief that this animal would be unusually valuable for studies in protein metabolism. This belief was strengthened by the observation that, when kept in a cage, a vigorous pig will take a sufficient quantity of a solution of starch containing the necessary salts, to meet all its energy requirements, day after day, with no evidence of anorexia and with no appreciable loss in weight. In an experience with more than a dozen animals, extending over a period of two and a half years I have found only two animals which proved unsatisfactory in this respect.

Preliminary to a series of experiments in which it was desired to feed quantities of nitrogen equivalent to the endogenous nitrogen metabolism of the animal, an examination was made of the possibility of using the creatinin excretion as an index to the amount of nitrogen derived daily from this source. It was believed that if the animals were given a liberal energy supply in the form of starch, and a salt mixture containing all the essential radicals, and water, he would ultimately reduce his exogenous protein metabolism to nearly, if not quite the vanishing point. In this condition the ratio of creatinin nitrogen to total nitrogen in the urine should become constant. If this constant ratio should be confirmed for a sufficient number of animals it would be a very valuable one. Such a ratio would make it possible to determine the nitrogen from endogenous metabolism. It would only be necessary to determine the creatinin in the urine for a number of days, to arrive at an average value, and multiply the nitrogen

appearing in this form by the factor derived from the constant ratio.

The results of experiments with seven animals are recorded in Table I. Since only the last portions of the records are of interest, only the following data are given. Body weight; length of the period on a nitrogen-free diet; average nitrogen content of the urine during the last five days; average content of creatinin nitrogen, and its ratio to the total nitrogen. It will be seen that the ratio of creatinin nitrogen to total nitrogen finally established is between 17.5 and 19 to 100. The average of all the experiments except the last is 18.5. The pig showing a ratio of 22 to 100 had been killed before the results were calculated, only the colorimeter readings having been recorded at the time of the experiments and the calculations made at a later date. It was therefore not possible to try this pig again. However, the ratios from the other animals fall within so narrow a range that their average 18.5 may be safely accepted as a close approximation to the final ratio on the level where the metabolism remains constant.

TABLE I. CREATININ N. AND TOTAL N. ELIMINATED, WITH N.-FREE DIET

The pigs were kept for long periods on N.-free diet and the ratio of Creatinin N. to Total N. eliminated in the urine was found.

No. of pig	Initial weight lbs.	Days on N.-free diet.	Av. N. content of urine last 5 days	Av. creatinin N. in urine last 5 days	Per cent of total N. as creatinin
16.....	24	27	.54	.104	19.19
21.....	85	24	1.83	.336	18.36
9.....	150.5	24	2.65	.471	18.05
8.....	43.5	36	1.09	.193	18.50
5.....	37	21	.90	.180	17.56
10.....	82	24	1.61	.314	19.17
7.....	165	23	2.61	.574	22.00

The animals lost but little weight in any case, and in a number the weight remained constant throughout the experiment. The time required to attain this ratio depends on the previous state of nutrition and on the volume of urine eliminated. A high output of urine tends to a more rapid attainment of the minimum level of nitrogen excretion. I have not observed the appearance of the above constant ratio in any case before the sixteenth day on a nitrogen-free diet.

The fact that a nearly constant ratio of creatinin nitrogen to total nitrogen is established under the conditions of these experiments does not, of course, necessarily mean that all of the nitrogen in the urine is derived from endogenous sources. An examination of several of these urines showed that about 60 per cent of the total nitrogen was still present in the form of urea.

Urea may also be a product of endogenous metabolic processes, or may result from the further decomposition of unknown bodies so derived. However this may be, the nitrogen eliminated when this nearly constant ratio is reached, represents the absolute minimum level of protein metabolism of which the animal is capable, and if nitrogen equilibrium is to be maintained, at least this amount of nitrogen must be supplied in the food and in a utilizable form. We are justified in assuming this nitrogen to be derived wholly from endogenous sources until further information is gained.

If we find the average amount of creatinin nitrogen appearing in the urine during four or five days when the pig is taking no food from animal sources, (that is a creatin- and creatinin-free diet) and multiply this by 5.5, the product will closely approximate the amount of nitrogen which the animal would eliminate daily in the urine if kept for a long period on a nitrogen-free diet. It is safe to accept this as the maintenance level.

Whether this factor will also apply to all species of animals as well as it does in the case of the pig, cannot be told at the present time, since no other species has as yet been investigated. Some investigators have not found the creatinin excretion as constant in the carnivora (dog) as has been found by Folin with men and by myself with the pig. Folin<sup>17</sup> has suggested that the endogenous metabolism of the carnivora may not be as constant as in other types of animals.

Another way in which the creatinin excretion will without doubt be of very great value in nutrition studies is in serving as a basis for the calculation of rations in animals employed in exact nutrition studies. In the past it has been

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<sup>17</sup> Folin, Amer. Jour. Physiol. 13, 84 (1905).

the custom of investigators to base the calculation of rations on the body weight of the animal. This method has always been known to have little merit, because of the great variation in the fat content of the body. In creatinin we have an end product of cellular activity and which is eliminated with surprising regularity, and while the amount eliminated is only roughly proportional to the body weight, it seems to be as Folin pointed out, closely proportional to the amount of metabolizing tissue in the body. If therefore, it is desired to feed two animals comparable quantities of a substance, as protein, it would undoubtedly be much more accurate to feed them each the same multiple of their respective creatinin nitrogen excretion. This method is being used in this laboratory in work now in progress on the efficiency with which the growing pig utilizes certain forms of nitrogen for growth.

It seemed to me worth while to study the relationship between the rise of creatinin elimination and the retention of nitrogen during growth in the pig. Data on this point have been collected with only three pigs and is presented here (Table II) without any expression as to finality since it may need revision in the light of further study.

TABLE II. N. RETENTION AND RISE OF CREATININ ELIMINATION

Relation between nitrogen retention and the rise of creatinin elimination in growing pigs.

Grams N. retained	Grams rise in creatinin N.	Grams N. retained corresponding to a rise of 1 mgm. of creatinin N.
175.....	.073	2.39
85.....	.039	2.18 <sup>a</sup>
329.....	.134	2.46

<sup>a</sup> The pig was sick at the close of this experiment and failed one day to void any urine, during the last six days from which the final creatinin output was estimated. This disturbance in the record renders the figure of very uncertain value.

NATURE OF THE REPAIR PROCESSES  
OF PROTEIN METABOLISM

E. V. McCOLLUM.

As a result of the great advances in our knowledge of the chemistry of the proteins, numerous problems relating to protein metabolism in the animal have arisen. It is now known that proteins from various sources differ widely from one another in fundamental characters, and that all are not equivalent as nutrients for animals. Casein and vitellin have been shown to be capable of maintaining an animal in nitrogen equilibrium, and recently Osborne, Mendel, and Ferry<sup>1</sup> have added glutenin from wheat to the list of proteins which are individually capable of meeting all the needs of an animal at least so far as maintenance is concerned, although up to the present time no very decided positive nitrogen balances have been reported in experiments where the animals were limited to a single protein as a source of nitrogen. Zein, gelatin and others have been found not to possess this power.

Prevailing views of the mechanism of protein metabolism have been supported and developed principally by Abderhalden. The amino acids, which all proteins yield on hydrolysis are regarded as the "building stones" with which the animal deals in constructing its specific body proteins. It is assumed that the animal has no synthetic power which enables it to produce from other complexes, the amino acids needed with the single exception of glycocoll. Henriques<sup>2</sup> has brought forward experimental evidence which conflicts with this view in that he kept rats in nitrogen equilibrium with a ration containing no protein other than gliadin, from which lysin is absent. Abderhalden<sup>3</sup> has disputed the possibility of accomplishing this with strictly pure gliadin.

From this view of the nature of protein metabolism it follows that the greater the similarity of the molecule of food protein to that of the specific body proteins, the greater will

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<sup>1</sup> Osborne, Mendel, and Ferry, Carnegie Inst. Bul. 156 (1911).

<sup>2</sup> Henriques, Ztschr. Physiol. Chem. 60, 105 (1909).

<sup>3</sup> Abderhalden, Ztschr. Physiol. Chem. 60, 425, 1909.

be the food value to the animal. It also follows that any one of the essential cleavage products which is present in smallest amount in food protein, determines the value of the entire molecule to the animal.

The most elaborate effort to test the validity of this hypothesis is the work of Michaud<sup>4</sup> who says "Mann nur dann dem Eiweissminimum am nächsten kommt, wenn man dass Körper-eigene Eiweiss verfüttert, das man sich aber umsomehr von diesem Minimum entfernt, als man ein in seiner chemischen Konstitution differentes Eiweiss gibt." If this is strictly true, a protein wholly lacking in one or more cleavage products found in the tissues of an animal should be entirely inadequate for the construction of new body protein when fed alone. Gelatin, gliadin and zein are in this class, yet all investigations with these proteins indicate that they are utilized as food by the animal even when fed as the sole source of nitrogen.<sup>5</sup> When fed in amounts corresponding to the fasting level of protein metabolism a considerable portion of the nitrogen fails to appear in the urine as promptly as do forms known not to be of value to the animal. This phase of the subject will be further treated when the results of Michaud are examined in some detail.

Michaud's method would appear in most respects to be a logical one for determining the value of a particular protein to an animal for purposes of repair of the tissue destroyed in endogenous metabolism. The plan is as follows: The animal is reduced to its lowest possible level of nitrogen elimination by feeding a nitrogen-free diet (starch, fat); and then an amount of nitrogen equivalent to the lowest daily output of which the animal is capable is fed in the form of the particular protein to be studied. The degree in which the animal utilizes the protein fed in replacing the tissue daily catabolized is taken as a measure of its value as a tissue-building material. The supposed efficiency of the method is based on the assumption that the animal will utilize the nitrogen presented to him for repair purposes as efficiently as it is possible to do so.

<sup>4</sup> Michaud, *Ztschr. Physiol. Chem.* **59**, 405 and 421 (1909).

<sup>5</sup> Compare Merlin, *Amer. Jour. of Physiol.* **19**, 285 and **20**, 234 (1907). Also Abderhalden, *Ztschr. Physiol. Chem.* **60**, 425 (1909); Henriques, *Ztschr. Physiol. Chem.* **60**, 105.



This assumes that it is possible for an animal to take a given amount of nitrogen and to convert it into one hundred per cent product, i. e. body tissue, provided its character is suitable. This assumption would seem to be supported by the experimental data available. Michaud found that when protein equivalent to the "protein minimum" was fed to dogs in the form of casein, or dog tissues, when the animals were metabolizing at their minimum level, there was no loss of nitrogen, which would seem to indicate a perfect utilization of protein at this low plane of intake.

The data presented in this paper are the outgrowth of a series of experiments undertaken two years ago, using essentially the method of Michaud, to compare the values of the protein mixtures of some of our most important grains as nutrients for the pig. It was hoped that specific differences would be shown by this method between grains in which the character of the protein mixture is known to differ so markedly as in the wheat, oat, and corn kernels.

#### PLAN OF EXPERIMENT

The animal (pig) was fasted for two or three days with water and salts until it would take a starch solution readily, and then it was given fifty calories per kilo per day in the form of starch together with a salt mixture having about the composition of the ash of the oat kernel. This mixture contained 15.5%  $K_2O$ , 3.0%  $Fe_2O_3$ , 3.3%  $CaO$ , 5.0%  $MgO$ , 25.0%  $P_2O_5$ , 4.0%  $SO_3$ , and 44.2%  $Cl$ .

The salts and starch were treated with a small amount of boiling water to scald a part of the starch, the mass quickly stirred and cool water added in amount to form a soup which the pig could drink readily. This ration was continued with daily quantitative collections of the urine and feces. The creatinin and total nitrogen were determined daily. From the amount of nitrogen eliminated during five to ten days in the form of creatinin, an average was obtained from which the nitrogen resulting from endogenous metabolism was calculated, using the formula 5.5 times creatinin nitrogen equals nitrogen from endogenous metabolism. (See page 78). The pig was then maintained on the nitrogen-free diet until the daily output of nitrogen in the urine reached about this level.

When the urinary nitrogen was entirely of endogenous origin for several days, as shown by a nearly constant ratio between the nitrogen as creatinin and the total nitrogen varying between 17:100 and 19:100, the pig was considered to be ready for an experiment in nitrogen feeding. The grain to be studied was now introduced into the ration and an isodynamic quantity of starch withdrawn. Since feeding a grain always leads to an increased excretion of nitrogen in the feces it was assumed that part of the nitrogen is not digested, and accordingly the amount of grain fed was such that if 90 per cent of the nitrogen were digested and absorbed, the absorbed part would just equal the amount of nitrogen degraded daily in endogenous metabolism. Actually the increased nitrogen in the feces amounted to somewhat more than 10 per cent of the amount fed so the amount absorbed was a little less than enough to meet the needs of the pig for repairs.

The results obtained were rather surprising. With the oat or corn kernel there was no appreciable change in the nitrogen content of the urine or in the ratio of the creatinin nitrogen eliminated to the total nitrogen. These remained essentially the same as in the latter part of the period when a nitrogen-free diet was given. With wheat a small rise in the nitrogen content of the urine was observed amounting to about ten to fifteen per cent. This difference was entirely too small to account for well known differences in the quantitative relations among the cleavage products of the wheat proteins and those of animal tissues thus far studied.<sup>6</sup> The gluten of wheat makes up about 75 per cent of the total protein content of the grain, and yields about 40 per cent of its nitrogen as glutaminic acid, while all animal proteins thus far examined contain less than 17 per cent of this complex. It is scarcely possible that the mixture of cleavage products obtained from the wheat grain should be suitable for rearrangement into muscle proteins with an efficient utilization of nitrogen. In all of these experiments with individual grains the efficiency of the collection of the urine was tested by giving one or more doses of urea to see if

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<sup>6</sup> Compare the papers of Osborne and his co-workers *Amer. Jour. Physiol.* **22**, 433, **23**, 81 (1908), **24**, 161 and 437 (1909). Also *Ergeb. Physiol. Abderhalden, Ztschr. Physiol. Chem.* **10**, 47 (1910), **51**, 311 and 404 (1907).

the nitrogen administered in this form could be traced into the urine. Urea nitrogen was invariably recovered promptly to the extent of 85 to 90 per cent in the urine *in excess of the endogenous nitrogen*, and such excess of nitrogen in the urine was accompanied by a fall of the per cent of total nitrogen in the form of creatinin. These results led me to undertake a series of experiments with pigs on the feeding of:

1. Zein nitrogen equivalent to the endogenous urinary nitrogen;

2. Zein nitrogen equivalent of two to nine times the endogenous nitrogen;

3. Gelatin nitrogen equivalent to the endogenous urinary nitrogen;

4. Casein nitrogen equivalent to twelve or thirteen times the endogenous urinary nitrogen.

The plan of these experiments differed from those of Michaud in certain respects. Michaud took as the "Eiweiss Minimum" the sum of the urinary and fecal nitrogen excreted by the animal after a long period on a nitrogen-free diet. I have taken the nitrogen of the urine alone as an index to the amount of nitrogen to be fed, and have interpreted the results largely on changes in the nitrogen content of the urine resulting from feeding the forms of nitrogen studied. The nitrogen of the feces is not in the form of end products of metabolism, but represents losses which might be termed accidental in character, such as cells abraded from the alimentary tract, the residues of the secretions which escape absorption, together with the bacterial growths for which these furnish a medium, as well as undigested remains of feed. These are all sources independent of the *essential tissue metabolism*. In experiments of this character we should deal specifically with the endogenous type of metabolism. The object should be to ascertain the influence of a particular protein on the repair of waste of an equivalent amount of tissue due to endogenous metabolism and not to attain a state of nitrogen equilibrium. The latter, to attain nitrogen equilibrium, would require enough new growth to replace those losses of accidental nature. The nitrogen of the feces may serve as an approximate indication of the thoroughness of absorption provided adequate records are obtained of a fore and after period on a nitrogen-free diet.

The importance of taking the urinary nitrogen as the minimum protein metabolism is evident because of the vitiating influence of feeding nitrogen at too high a level in this class of work. It is evident that if we are to measure the efficiency of a particular protein by the excessive excretion of nitrogen during a feeding period, over the excretion on a nitrogen-free diet, it is imperative that the amount of nitrogen fed shall not exceed the urinary nitrogen of endogenous origin. If any excess over this amount is given, the animal is no longer limited by the one essential cleavage product present in smallest quantity. He is given some choice among the group of "building stones" larger than is necessary for repair.

Michaud makes no mention of giving his dogs an adequate supply of inorganic salts. In some of his feeding periods a certain amount of salts were carried by the protein mixture given, but when pure proteins were fed the dogs were apparently taking a nearly salt-free ration. In my own experiments a liberal supply of a salt mixture was given daily. The amount of water given was large enough to keep the volume of urine rather high. It was believed that this tended to a more prompt, uniform and complete elimination of the end products of metabolism.

The investigation of this subject has extended over two years, and a very large amount of data has been collected. The tables here presented show representative protocols. All the data collected were essentially in harmony. The pigs used were from the Wisconsin Experiment station herd, all were of the larger breeds, and were of the best growing strains. Animals found to be infested with intestinal parasites were discarded.

An examination of Tables I and II shows that when a pig has been reduced by a long continued nitrogen-free diet, to a condition where the exogenous type of metabolism has probably entirely disappeared, the addition of an amount of nitrogen as zein closely approximating that derived daily as end products from endogenous metabolism, the rise in the nitrogen content of the urine is very much less than one would expect for a protein of this character. Although three amino acids are lacking: glycocoll, tryptophane and lysin, all of general occurrence in animal tissues, it would seem evident that the animal

has made use of a large part of the nitrogen of this protein. The ratio of creatinin nitrogen to total nitrogen falls but little from what it is on a nitrogen-free diet which shows that the nitrogen of the urine is derived principally from endogenous

TABLE I. FEEDING ZEIN EQUIVALENT TO ENDOGENOUS METABOLISM

Weight at beginning of experiment 37 pounds (16.82 kilograms).  
Pig was placed in the cage March 11, and fed starch, salt mixture and water during the following twenty days. No record of the nitrogen output until March 23.

Date	Grams N. in food	Grams N. in urine	Grams N. in feces	Grams N. as creatinin	Per cent of total N. as creatinin.
March	Starch				
28.....	0	.96	.24	.164	17.08
27.....	0	.94	.24	.143	15.21
28.....	0	.89	.24	.173	19.05
29.....	0	.86	.24	.165	18.18
30.....	0	.84	.24	.143	17.02
31.....	0	.96	.24	.154	15.10
		Av. .91 gr.			
April	Starch, zein				
1.....	.47	.95	.30	.166	17.46
2.....	.95	1.19	.30	.149	12.52
3.....	.95	.96	.30	.144	15.00
4.....	.95	1.10	.30	.143	13.00
5.....	.95	1.08	.30	.168	15.37
6.....	.95	1.13	.30	.141	12.47
7.....	.95	.90	.30	.139	15.44
8.....	.95	1.24	.30	.185	14.92
9.....	.47	1.01	.30	.157	15.54
	Starch				
10.....	0	1.00	.28	.157	15.70
11.....	0	.83	.28	.154	18.55
12.....	0	.61	.28	.122	19.99
13.....	0	.81	.28	.133	16.42
14.....	0	.77	.28	.165	21.42
	Starch, urea				
15.....	1.00	.80	.28	.147	18.37
16.....	1.00	1.27	.28	.157	12.36
17.....	1.00	1.52	.28	.132	8.66
18.....	.50	1.59	.28	.156	9.81
	Starch				
19.....	0	1.21	.28	.162	13.39
20.....	0	1.53	.28	.151	9.86
21.....	0	1.23	.28	.141	11.47
22.....	0	.89	.28	.160	17.97

sources. The second starch period eliminates the possibility of a lag in the excretion of the zein nitrogen. The urea period following the second starch period shows how quickly the composition of the urine changes when a form of nitrogen useless to the animal is introduced. The total nitrogen rises at once and with this goes a marked change in the per cent of the total nitrogen in the form of creatinin. Reference will be made later to the influence of similar amounts of gelatin nitrogen when administered to an animal under these conditions.

It has been customary with many workers in interpreting the new data in experiments for determining the nutritive value of proteins, to judge from a plus or minus nitrogen balance as

to whether the protein in question is sufficient or insufficient to maintain the animal in nitrogen equilibrium. We should go farther than this. Looked at in this way the protocols shown in Tables I and II both show decided nitrogen deficits. If the nitrogen content of the feces is enough greater in the period

TABLE II. FEEDING ZEIN EQUIVALENT TO ENDOGENOUS METABOLISM

Weight of pig at beginning of experiment 158 pounds (71.8 kilograms).  
 December 11 to 17 fed 50 calories per kilo as starch, with salt mixture and water.  
 Urine not examined.  
 During the four days' fast the animal was given two grams of agar-agar daily to insure regular evacuations. The feces were collected from December 14 to 21 inclusive, no agar-agar being given. The feces for December 21 were marked off by a fifteen-gram dose  $\text{Ca}_3(\text{PO}_4)_2$ . The nitrogen content for this period was 6.16 grams or .77 grams per day. The remainder of the protocol is given in tabular form.

Date	Grams N. in food	Grams N. in urine	Grams N. in feces	Grams N. as creatinin	Per cent. of N. as creatinin
December.	Starch				
18.....	0	2.59	.77	.48	18.64
19.....	0	2.61	.77	.44	17.01
20.....	0	2.66	.77	.50	18.94
21.....	0	2.58	.77	.48	18.83
	Starch, zein				
22.....	2.62	2.98	.72	.45	15.10
23.....	2.62	2.48	.72	.49	18.77
24.....	2.62	2.75	.72	.47	17.09
25.....	2.62	2.97	.72	.52	17.51
26.....	2.62	2.62	.72	.48	18.32
27.....	2.62	2.56	.72	.39	15.23
28.....	2.62	3.87	.72	.70	18.09
29.....	2.62	3.11	.72	.48	15.43
30.....	2.62	3.86	.72	.55	14.28
31.....	2.62	3.07	.72	.48	15.63
January					
1.....	1.31	2.37	.72	.19	16.45
	Starch				
2.....	0	3.02	.72	.46	15.20
3.....	0	2.43	.68	.52	21.40
4.....	0	2.94	.68	.46	15.64
5.....	0	2.67	.68	.48	17.97
	Starch, urea				
6.....	2.62	2.58	.68	.48	18.60
7.....	2.62	4.90	.68	.48	9.75
8.....	0	5.04	.68	.52	10.31
9.....	0	3.01	.68	.47	15.61
10.....	0	2.70	.68	.48	17.40
11.....	0	2.66	.68	.48	18.04

when the protein is fed, than it was during the last portion of the nitrogen-free period to indicate with certainty an incomplete digestion and absorption of the food protein, the *increase* in the fecal nitrogen should be considered as an index to the degree of absorption. But knowing the amount of nitrogen absorbed, our judgment concerning its value for replacing the endogenous loss should be based entirely on changes observed in the urine viz., on the increase if any, in the total nitrogen, and the change in the ratio between the nitrogen as creatinin and the total nitrogen. Looked at from this standpoint, the

tables give an accurate idea of the nutritive value of the nitrogen of zein for repair purposes.

During the nine days when zein was fed (Table I) the pig would have lost 8.19 grams ( $9 \times .91$ ) of nitrogen if he had been kept on a starch diet. He was fed 7.59 grams of nitrogen as zein and his total output of nitrogen in the urine during these nine days was 9.61 grams which is only .16 gram more per day than he would have excreted if no nitrogen had been given. The deficit of urinary nitrogen for the nine days was only 1.4 grams. It would seem therefore that the 7.59 grams of nitrogen fed had replaced 6.77 grams of this element lost through endogenous metabolism in this period.

An inspection of Table II shows that during the eleven days of zein feeding the output of nitrogen in the urine was 32.6 grams, whereas the nitrogen output on a starch diet as calculated from the creatinin output during the same time as observed in the preliminary starch period would have been 28.8 grams. During this period 27.5 grams of nitrogen were fed as zein. The loss of nitrogen by the pig was thereby reduced from 28.8 to 3.8 grams during the eleven days. Since, however, in the starch period following, .58 grams of nitrogen was excreted in excess over what was to be expected, the loss should be considered about 4.5 grams. It would appear that, although zein is lacking in three amino acids, its efficiency as a repair material in cellular metabolism is quite high.

The creatinin output during the first ten days on a starch diet averaged .201 gram of nitrogen per day. From this the nitrogen equivalent to the endogenous metabolism of the animal was calculated to be 1.105 grams. ( $.201 \times 5.5$ ). The total nitrogen in the urine was not determined during the first five days. The nitrogen of the urine had not fallen to the lowest possible level at the end of ten days on a nitrogen-free diet, as shown by the value of the endogenous metabolism calculated from the creatinin output, and also by the fact that the nitrogen appearing as creatinin was still only about 16 per cent of the total. It was assumed, however, that the pig was sufficiently freed from the end products of metabolism for the purpose of this experiment which was to determine whether an appreciable retention of nitrogen could be induced by feeding zein in excess of the "maintenance" requirements of the animal.

All previous efforts to induce growth in animals by feeding zein as the sole source of protein have failed entirely<sup>7</sup> but as will appear later in this paper the pig appears to be exception-

TABLE III. FEEDING ZEIN ABOVE NEEDS FOR REPAIR

Record of a pig on a ration supplying nitrogen in excess of the needs of the animal for repair, in which zein was the only source of protein. Weight of pig at beginning of experiment 63.5 pounds (28.86 kilograms). Weight at end of experiment 64.5 pounds (29.33 kilograms).

Date	Grams N. in food	Grams N. in urine	Grams N. in feces	Grams N. in urine as creatinin
June	Starch			
29.....	0	1.78	.59	.201
30.....	0	1.70	.59	.189
July				
1.....	0	1.42	.59	.194
2.....	0	1.34	.59	.223
3.....	0	1.29	.59	.210
	Starch, zein			
4.....	1.78	1.20	.57	.204
5.....	1.78	1.40	.57	.200
6.....	1.78	2.19	.57	.197
7.....	1.78	1.83	.57	.194
8.....	1.78	1.73	.57	.174
9.....	1.78	1.79	.57	.174
10.....	1.78	1.95	.57	.214
11.....	1.78	1.99	.57	.189
12.....	1.78	2.13	.57	.199
13.....	1.78	2.13	.57	.195
14.....	2.23	2.04	.61	.214
15.....	2.23	2.25	.61	.206
16.....	2.23	3.23	.61	.185
17.....	2.23	1.08	.61	.194
18.....	2.23	2.43	.61	.192
19.....	2.23	2.52	.61	.234
20.....	2.23	2.38	.61	.201
21.....	3.35	2.16	.66	.204
22.....	3.35	2.80	.66	.182
23.....	3.35	2.16	.66	.182
24.....	3.35	3.78	.66	.195
25.....	3.35	2.89	.66	.214
26.....	3.35	2.98	.66	.200
27.....	3.35	2.82	.66	.170
28.....	3.35	3.21	.66	.196
29.....	3.35	2.95	.66	.209
30.....	3.35	3.14	.66	.228
31.....	0	2.96	.66	.199
August	Starch			
1.....	0	2.64	.66	.204
2.....	0	2.64	.66	.210
3.....	0	1.61	.66	.....
4.....	0	1.62	.66	.....
5.....	0	1.08	.66	.....
6.....	0	1.29	.66	.....

ally efficient in the utilization of food-stuffs so it appeared possible that more favorable results might be met in the case of zein. The record given in Table III begins with the sixth starch day.

Table III shows the behavior of a pig on a ration supplying zein in quantities as high as three times greater than the endog-

<sup>7</sup> Willcock and Hopkins, Jour. Physiol. **35**, 117 (1906-7). Henriques, Ztschr. Physiol. Chem. **60**, 425 (1909).



enous metabolism. If we consider the record beginning July 4 and ending with the experiment (34 days), the nitrogen fed was 66.9 grams. The total elimination in the urine of the entire period was 76.7 grams of nitrogen leaving a negative balance of 9.8 grams of nitrogen in the urine. The nitrogen content of the feces in the periods separated shows that the digestion was complete. The after period on starch was made long enough to bring the pig to the same level of nitrogen elimination as at the beginning of the feeding of zein. We must consider, however, that if the pig had received no protein during these 34 days (starch diet) the nitrogen elimination in the urine would have been about 37.4 grams. By feeding zein the loss of nitrogen from endogenous metabolism was reduced by 27.6 grams. That is 27.6 grams of this element degraded through tissue catabolism was repaired from the zein fed. Of the excessive nitrogen fed, however, none was stored as gain. The same care was given to the collection of the excreta as in the case of other animals in experiments in which the addition of a small amount of urea to the diet led to a prompt detection of a corresponding rise in the nitrogen excreted. The above difference is too large to be charged to experimental error.

It might be urged that the amount of zein fed in this case was too small in excess of the maintenance needs to be conducive to growth. It is true, we know almost nothing of the influence of the plane of protein intake on the tendency of the growing animal to construct new body protein. Table IV is therefore presented giving the record of a pig which took a relatively high protein diet in which zein furnished all the nitrogen.

The pig from which the data given in Table IV were obtained weighed 51 pounds (23.18 kilograms) at the beginning and lost one pound during the experiment. He was fed a starch and salt diet (50 calories) during an eleven day fore period, during which the average nitrogen output in the form of creatinin was .19 grams daily. Assuming that he would finally have established a ratio of 18.5:100 between creatinin nitrogen and total nitrogen, the endogenous metabolism of this pig was 1.04 grams of nitrogen daily. This level was not reached in eleven days, the nitrogen content of the urine on

the tenth and eleventh days of the fore period being 1.37 and 1.41 grams respectively. However, it was believed that the animal was in a satisfactory condition for an experiment of this character. He was thereafter fed zein as indicated in Table IV.

TABLE IV. NITROGEN EXCRETION FROM HIGH INTAKE OF ZEIN

Date	Grams N. in food	Grams N. in urine	Grams N. in feces
<b>February</b>			
1.....	4.47	1.41	.74
2.....	4.47	2.35	.74
3.....	4.47	3.27	.74
4.....	6.59	4.35	.74
5.....	8.82	4.23	.74
6.....	9.24	8.46	.74
7.....	9.53	8.28	.74
8.....	9.53	8.44	.74
9.....	9.53	8.13	.74
10.....	9.53	8.23	.74
11.....	9.53	8.18	.74
12.....	9.53	7.43	.74
13.....	9.53	8.23	.74
14.....	9.53	8.55	.74
15.....	9.53	8.60	.74
16.....	9.53	7.96	.74
17.....	9.53	8.60	.74
	<b>Starch</b>		
18.....	0.	8.62	.74
19.....	0.	6.04	.74
20.....	0.	4.78	.74
21.....	0.	3.70	.74
22.....	0.	2.34	.64
23.....	0.	2.25	.64
24.....	0.	1.45	.64
25.....	0.	1.45	.64
26.....	0.	1.28	.64
<b>Total.....</b>	<b>142.89</b>	<b>146.59</b>	<b>18.74</b>

The record in Table IV shows a marked utilization of zein nitrogen in supplying material for the replacement of nitrogen lost through endogenous metabolism. Although the table as a whole shows a negative balance, it is by no means great enough to indicate a total lack of usefulness of zein nitrogen.

During the first seventeen days of the experiment the pig was fed 142.28 grams of nitrogen. On account of the lag in the nitrogen excretion it is necessary to consider the feeding period and the starch period following, together. During the twenty-six days covered by these periods the nitrogen excreted in the urine was 150.59 grams. The total deficit was, therefore 8.31 grams. If the pig had been kept on a starch diet he would, judging from the creatinin output, have lost 27.04 grams of nitrogen from endogenous metabolism. If none of the zein had been utilized by the pig he should have excreted

in his urine all of the nitrogen taken in this form together with the 27.04 grams derived from endogenous sources. The nitrogen elimination in the urine should, under these circumstances have been  $142.28 + 27.04 = 169.32$  grams for the twenty-six days. The difference between this figure and the nitrogen found in the urine ( $169.32 - 150.59 = 18.73$ ) we are to suppose was replaced from the zein fed. No new growth has been demonstrated in any case when zein has been the only protein supplied. These results with zein were so unexpected in character that it was thought desirable to make similar experiments with gelatin, using the pig, in order to compare the efficiency of this species, with others in the utilization of this form of nitrogen. Gelatin has been used in nutrition studies more than any of the other "incomplete" proteins. In most of the recorded experiments it has seemed doubtful whether the animals were fed gelatin nitrogen at the same level as the endogenous metabolism, and that this fact would account for the lack of uniformity in the results of various observers, as to the nutritive value of this substance. Accordingly six experiments were carried out on the same plan as used in the experiments recorded in Tables I and II. For presentation here, the record is selected of a pig fed starch during a long fore period, then zein, followed by a starch period, then gelatin at the same level as zein, and lastly a starch period long enough to allow the pig to return to his endogenous level. (See Table V.) The results were all in harmony and showed an apparent utilization of between 50 and 60 per cent of the nitrogen given in this form for purposes of repair. The utilization of gelatin nitrogen fell in all cases far short of that of zein. Not more than 20 per cent of the nitrogen fed as zein has ever been traced into the urine in excess of the endogenous output, when the amount fed did not exceed 5.5 times the nitrogen daily eliminated by the animal as creatinin. When gelatin was fed in like amounts, between 40 and 50 per cent of the nitrogen given was promptly recovered in the urine in excess of what should have been found had a nitrogen-free diet been taken during the same period.

When considering experiments like that shown in Table IV, we are confronted by the fact that no very decided positive nitrogen balance has as yet been obtained in experiments

where but one protein has been given. I have reported experiments in which an appreciable growth was secured in young rats fed a mixture of two proteins, together with carbohydrates, fats and the necessary inorganic salts.<sup>s</sup> These are the most successful of any yet reported, with so simple a ra-

TABLE V. ZEIN AND GELATIN EQUIVALENT TO ENDOGENOUS METABOLISM

Weight of pig 150.5 pounds at beginning; 152 pounds at end of experiment.

The pig was fed starch (90 calories per kilogram), a salt mixture, and water during a twenty-four day period. A quantitative record was kept daily of the nitrogen content of the urine, and feces and also of the creatinin output. The record presented begins with the twentieth day on a nitrogen-free diet.

Date	Grams N. in food	Grams N. in urine	Grams N. in feces	Grams N. as creatinin	Per cent of total N. as creatinin
Dec.	Starch				
17.....	0	2.54	.94	.476	18.74
18.....	0	2.59	.94	.485	18.72
19.....	0	2.64	.94	.474	18.16
20.....	0	2.92	.94	.465	15.92
21.....	0	2.59	.94	.475	18.34
	Starch zein				
22.....	2.62	1.98	1.06	.325	16.40
23.....	2.62	2.48	1.06	.516	20.80
24.....	2.62	2.75	1.06	.574	20.87
25.....	2.62	2.98	1.06	.425	14.26
26.....	2.62	2.62	1.06	.465	17.74
27.....	2.62	2.56	1.66	.473	18.47
28.....	2.62	3.87	1.06	.494	12.78
29.....	2.62	3.11	1.06	.481	15.46
30.....	2.62	3.85	1.06	.476	12.36
	Starch				
31.....	0	3.07	1.06	.472	12.36
Jan.					
1.....	0	2.37	1.06	.473	19.95
2.....	0	3.69	1.06	.....	.....
3.....	0	2.43	1.06	.481	19.79
4.....	0	2.94	1.06	.471	16.00
	Starch gelatin.				
5.....	2.62	3.02	1.06	.474	15.69
6.....	2.62	3.15	1.04	.465	14.73
7.....	2.62	3.78	1.04	.473	12.51
8.....	2.62	4.32	1.04	.475	10.99
9.....	2.62	3.20	1.04	.472	14.75
10.....	2.62	5.57	1.04	.492	8.83
11.....	2.62	3.46	1.04	.476	13.75
12.....	2.62	3.24	1.04	.474	14.62
	Starch				
13.....	0	3.02	1.04	.....	.....
14.....	0	3.35	1.04	.....	.....
15.....	0	2.60	1.04	.....	.....
16.....	0	3.00	1.04	.....	.....
17.....	0	2.40	1.04	.....	.....

tion. The growth was in no case comparable to normal growth in the rat and the high degree of uncertainty, and general unsatisfactoriness of the results, render this line of experimentation very discouraging as a means of throwing light on the chemical processes of metabolism. However, the impor-

<sup>s</sup> Amer. Jour. Physiol. 25, 120 (1909).

tance of finding a mixture of pure chemical substances on which an animal will grow in a nearly normal way is great and warrants the expenditure of much effort in this direction.

In feeding rations compounded of relatively pure substances my attention has been repeatedly attracted by the marked differences exhibited by individuals of the same species, in their behavior toward such mixtures. Osborne and Mendel have called attention to the same thing in their work.<sup>9</sup> This fact together with a remarkable difference in the attitude of animals of different species toward rations made up of naturally occurring mixtures but derived from restricted sources, has been strongly emphasized in my own experience. Of twenty-four young rats ranging from 30 to 70 grams in weight, when fed on wheat alone, but nine made gains of 15 grams or more during the first three months. Eleven made no gains at all, but died in forty to sixty days having suffered no appreciable change in weight during that time. Only two doubled their weight on this ration. All of these rats if fed a normal ration should have attained weights of from 190 to 225 grams in this period. This happened, not in a single experiment, but in three experiments in three different years. In a similar trial with three young pigs of fifty pounds weight, in which the wheat kernel was the only food given, and the animals were kept away from vegetation, almost normal growth was observed during the first six months, and in the case of one animal with which the ration was continued nine months a weight of 190 pounds was attained. This observation together with the knowledge that a pig will readily take a sufficient quantity of a starch solution to cover his energy requirements, and will continue to do so for at least thirty-five days with no evidence of anorexia and no appreciable loss in weight led me to try the pig on a mixture of casein, starch, salts and water. The results were gratifying and have convinced me that the pig is particularly suitable for this class of work. The records of two pigs kept in metabolism cages and fed this simple mixture, are shown in Tables VI and VII. It will be seen on inspecting Table VI that a pig of 48 pounds began to retain nitrogen at a remarkable rate and continued to do so at a perfectly satis-

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<sup>9</sup> Osborne and Mendel, Carnegie Inst. Bul. 156 (1911).

factory rate during a period of thirty-six days. On February 13 it was noticed that the pig was not as eager to be let into the feeding stall as he usually was, but he entered of his own accord and ate all of his food. From that time on he grew

TABLE VI. FEEDING CASEIN AS THE ONLY SOURCE OF PROTEIN

Weight of pig 48 pounds (21.81 kilograms) at beginning, 55 pounds (25 kilograms) at end of experiment. The pig was allowed to go two days with water alone, and was then fed 100 calories per kilogram as starch, an inorganic salt mixture with additional ground rock phosphate and casein as indicated in the table.

Date	Grams N. in food	Grams N. in urine	Grams N. in feces	Total grams of N. output	Grams N. as creatinin
<b>Jan.</b>					
13.....	14.15	2.85	.60	3.45	.230
14.....	14.15	3.00	.60	3.60	.266
15.....	14.15	5.86	.60	6.46	.247
16.....	14.15	5.31	.60	5.91	.234
17.....	14.15	5.75	.60	6.35	.236
18.....	14.15	5.07	.60	5.67	.266
19.....	14.15	5.09	.60	5.69	.271
20.....	14.15	5.53	.60	6.13	.243
21.....	14.15	5.51	.60	6.11	.258
22.....	14.15	5.79	.60	6.39	.272
23.....	14.15	5.27	.60	5.87	.291
24.....	14.15	5.83	.60	6.43	.243
25.....	14.15	4.70	.60	5.30	.278
26.....	14.15	6.11	.60	6.71	.279
27.....	14.15	6.39	.60	6.99	.284
28.....	14.15	6.39	.60	6.99	.293
29.....	14.15	6.09	.60	6.69	.248
30.....	14.15	6.17	.60	6.77	.289
31.....	14.15	6.43	.60	7.03	.286
<b>Feb.</b>					
1.....	14.15	5.90	.60	6.50	.293
2.....	7.10	7.93	.60	8.53	.324
3.....	7.10	8.70	.60	9.30	.285
4.....	10.66	9.04	.60	9.64	.277
5.....	14.21	7.67	.60	8.27	.320
6.....	14.21	7.50	.74	8.24	.302
7.....	14.21	9.00	.74	9.74	.284
8.....	14.21	8.04	.74	8.78	.287
9.....	14.21	8.39	.74	9.13	.325
10.....	14.21	8.31	.74	9.05	.290
11.....	14.21	8.16	.74	8.90	.311
12.....	14.21	7.77	.74	8.51	.328
13.....	14.21	9.57	.74	10.31	.307
14.....	14.21	9.66	.74	10.40	.272
15.....	14.21	9.92	.74	10.66	.330
16.....	14.21	9.75	.74	10.49	.355
17.....	.....	9.82	.74	10.56	.312
<b>Total..</b>	<b>478.38</b>	<b>248.27</b>	<b>23.28</b>	<b>271.55</b>	

The pig died February 17.

Nitrogen retained in the thirty-six days..... 206.83 grams

Average creatinin N. during the first five days..... .242 grams

Average creatinin N. during the last five days..... .315 grams

Increase..... .073 grams

more listless, but his appetite remained good and he ate all of the food. The nitrogen output had been gradually increasing after the first two weeks. On the evening of February 16, when he started to the feeding stall to eat, he fell and could

not get up. He seemed to have lost the use of his legs, especially the hind legs. He was evidently in great pain for he squealed loudly and trembled all over. The muscles were relaxed during this attack. After a quarter of an hour the evi-

TABLE VII. FEEDING CASEIN AS THE ONLY SOURCE OF PROTEIN

Ration: casein, starch, ash of milk, NaCl and water.

Initial weight 43.5 pounds; final weight 51 pounds.

This pig was kept on a starch diet (N-free) from March 11 to April 16.

The average creatinin output during the first ten days on starch diet was .191 gram N. Only the last twelve of this period are included in the table.

Began feeding casein April 16.

Date	Grams N. in food	Grams N. in urine	Grams N. in feces	Grams total N. output	Grams N. as creatinin
April	Starch				
4	0.	1.64	.34	1.98	.204
5	0.	.76	.34	1.10	.188
6	0.	1.24	.34	1.58	.174
7	0.	1.07	.34	1.41	.200
8	0.	1.12	.34	1.46	.189
9	0.	1.01	.34	1.35	.205
10	0.	1.12	.34	1.46	.193
11	0.	1.10	.34	1.44	.207
12	0.	1.02	.34	1.36	.184
13	0.	1.21	.34	1.55	.191
14	0.	1.02	.34	1.36	.192
15	0.	.91	.34	1.25	.....
16	Casein, starch				.....
17	6.86	.99	.39	1.38	.....
18	13.72	2.22	.39	2.61	.....
19	13.72	2.67	.39	3.06	.....
20	13.72	5.85	.39	6.24	.....
21	13.72	4.14	.39	4.53	.....
22	13.72	4.77	.39	5.16	.....
23	13.72	4.20	.39	4.59	.....
24	6.86	5.28	.39	5.67	.219
25	13.72	5.41	.39	5.80	.203
26	6.86	4.29	.39	4.68	.234
27	13.72	11.33	.39	11.72	.212
28	10.97	9.46	.39	9.85	.....
29	10.97	11.01	.39	11.40	.....
30	10.97	8.34	.39	8.73	.....
May	10.97	6.53	.39	6.92	.....
1	10.97	5.66	.39	6.05	.....
2	10.97	3.63	.39	4.02	.....
3	10.97	5.53	.39	5.92	.....
4	10.97	4.19	.39	4.58	.246
5	10.97	3.41	.39	3.80	.251
6	0.	0.	.39	.39	.....
7	5.48	5.43	.39	5.82	.367
8	8.22	3.38	.39	3.77	.253
9	16.97	8.11	.39	8.50	.262
Total	259.74	139.05	13.44	152.49	.....

Average output of nitrogen as creatinin during the last twelve days on a starch diet .189 grams per day. Output during the last six days of casein feeding .228 grams per day.

dence of pain disappeared, but the animal was still almost helpless. When helped to the trough he ate all of his food and was helped back again to the cage. The next day he seemed to rest easily and ate a portion of his food when assisted to the trough. When left alone, he lay flat on his side with the legs extended but not rigid. The muscles were all relaxed and the reflexes

were all present. When helped to his feet he squealed constantly and shifted rapidly from one hind foot to the other, turning around slowly all the time. When assisted to get off his feet, which he seemed unable to do himself, he assumed the posture described above, became quiet and was apparently much easier. He appeared no worse during the day, but at five o'clock, when urged to enter the cage he fell again squealing loudly, and died an hour later.

Autopsy revealed a marked congestion of the entire digestive tract, but much more pronounced in the posterior half. The colon was packed with putty like feces. Conical necrotic areas were found on both kidneys. Dr. Hadley, Station Veterinarian, suggested acute poisoning, but an examination of the salt mixture failed to reveal the presence of any toxic metal as was suspected. The casein used in this experiment was made by the method of Hammarsten, from separator skim milk from the dairy building of the Experiment Station, and all vessels used were glass.

The experiment was repeated on another pig using ash of whey instead of the salt mixture made up from reagent bottles. The same calcium phosphate was given as was used in the first experiment, to give increased body to the feces. The second experiment was made with a pig which had been thirty-six days on a nitrogen-free diet. On the twenty-fourth day of casein feeding, the pig fell into the same condition as the first, but was saved by promptly feeding whole milk and rolled oats. Food had to be introduced into the mouth at first, but after three days he was on his feet again and after two weeks on the regular farm ration, in an open lot, he had gained 12.1 pounds. When the pig was fully recovered he was again placed on a starch ration and used in a long continued experiment in low protein feeding.

It is scarcely warrantable to attribute the acute attacks of congestion which came upon these pigs, to the casein fed. Osborne and Mendel report having kept rats on a similar ration for a period of 160 days without serious disturbances. The difficulty with these pigs is probably to be attributed to the peculiar conformation of the colon in the pig, and to impaction with feces of unfavorable character. This point is being further investigated.



Table VI shows a positive nitrogen balance of 206.8 grams. The stomach contained some liquid and the intestine a considerable quantity of fecal matter, and the tissues of the animal some end products and partly metabolized nitrogenous substances. Experience with other animals leads me to believe that this pig's body contained about 25 grams of nitrogen which, if he had been placed on a starch diet, would have appeared in the urine before the minimum level was reached. It would seem therefore that the pig had utilized for the construction of new body tissue about 175 grams of nitrogen taken as casein. With this retention of nitrogen there was a gradual rise in the output of creatinin from .242 grams per day during the first five days, to .315 grams during the last five days.

The pig whose record is shown in Table VII had, when taken ill at the end of the experiment, eaten 259.74 grams of nitrogen, and<sup>1</sup> had excreted during the same time 152.49 grams. Since this pig was excreting only about 4 to 5 grams of nitrogen daily in the urine it is probable that he would have returned to the minimum level of nitrogen excretion, had he been placed on a starch diet, with a loss of about fifteen grams of nitrogen. This pig had retained and converted into body tissue about 80 to 85 grams of casein nitrogen. It is unfortunate that the creatinin record was disturbed by the failure of the pig to eat or void any urine May 6. This makes it impossible to arrive at a very satisfactory figure for creatinin at the end of the experiment. The average for the beginning was .189 grams and for the last six days, after feeding casein .228 grams per day.

If we can judge from the change in the creatinin elimination, as to the body content of protoplasm, we must conclude that these pigs increased their metabolizing tissue by an amount equivalent to one-fifth to one-fourth on a diet containing but a single protein. This is without doubt the best evidence yet produced, of the *chemical* sufficiency of casein for both maintenance and growth. These experiments with casein greatly increase the value of the negative results obtained in the attempts to induce growth by feeding zein as the only protein.

#### DISCUSSION OF RESULTS

As before stated, the experiments reported in this paper were planned to give an answer to the question: Why is the

procedure of reducing an animal to its lowest possible level of protein metabolism by feeding a starch diet, then feeding an amount of nitrogen in the form to be studied equivalent to the endogenous metabolism and observing the excessive elimination of nitrogen in the urine, not a method for comparing the relative values of proteins as nutrients for that species? All of my observations have led to the belief that zein or gelatin when supplying the only source of nitrogen are useful in an important degree, to the animal in replacing the nitrogen lost through endogenous metabolism. These "incomplete" proteins when fed in liberal amounts do not seem to supply all the complexes necessary for growth. The results of Michaud with gliadin are substantially in harmony with mine for zein. In his gliadin period Michaud fed 1.42 grams of nitrogen daily. The average negative balance during 9 days was .350 grams nitrogen, equivalent to 24.64 per cent of the nitrogen fed. In his other gliadin periods the negative balances were between 15.05 and 36.33 per cent of the nitrogen fed. Murlin<sup>10</sup> found gelatin to be utilized by dogs to the extent of 31 per cent of the nitrogen given. My results differ from his only in their greater uniformity due to the fact that my animals were reduced to their endogenous level of metabolism in the fore period whereas it seems very doubtful whether any of his were in this condition. Even with a liberal daily elimination of urine I have not observed pigs to reach this level on a nitrogen-free diet in less than sixteen days.

The fact that certain proteins, lacking in one or more cleavage products known to be necessary to the formation of the proteins of the animal body are of relatively high efficiency in preventing loss of body nitrogen due to endogenous metabolism, yet are insufficient for growth, forces one to the conclusion that the processes of replacing nitrogen degraded in cellular metabolism are not of the same character as the processes of growth. It seems also to be a necessary conclusion that the processes of cellular catabolism and repair do not represent a series of chemical changes involving the destruction and reconstruction of an entire protein molecule. This idea does not

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<sup>10</sup> Murlin, Amer. Jour. of Physiol. 20, 240 (1907).

conflict with the theory of protein metabolism offered by Folin.<sup>11</sup> Osborne and Mendel<sup>12</sup> have recently shown that certain proteins may support an animal in nitrogen equilibrium for long periods and yet be unable to produce growth. My experience in feeding casein as the only protein has convinced me that a marked increase in the protein content of the body may result from taking a single protein in the food. The facts therefore lead to the belief that in order that an animal may grow, the food protein must supply complexes not necessary for the endogenous upkeep.

It is interesting to correlate the results of these experiments with the findings of Folin<sup>13</sup> in his studies of the fate of creatin when fed to men on a low protein diet. He was unable to trace the nitrogen of this complex into the urine under these conditions and was led to conclude that creatin acts as a food and not as an end product of metabolism.

These fragments of evidence do not harmonize with the views of Michaud,<sup>14</sup> which have been endorsed by Aberhalden.<sup>15</sup> In fact it seems rather surprising that Michaud should draw the conclusion quoted early in this paper when he could trace but 15 to 36 per cent of the nitrogen of gliadin into the urine in his feeding experiments with this protein known to be lacking in one cleavage product, lysin, present in all animal proteins examined. According to his theory this protein should be without value to the animal, so far as its nitrogen is concerned, if fed alone.

#### SUMMARY OF CONCLUSIONS

Attention is called to the fact that the results of experiments in feeding the mixture of proteins occurring in individual grains, in quantity equivalent to the lowest possible level of protein metabolism of which the animal is capable, do not indicate as wide differences in the nutritive values of the protein of the wheat, oat, and corn kernels as would be expected from the known chemical differences in these proteins.

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<sup>11</sup> Amer. Jour. Physiol. **13**, 117 (1905).

<sup>12</sup> Carnegie Inst. Bul. 156 (1911).

<sup>13</sup> Festschrift für Olaf Hammarsten, 1906.

<sup>14</sup> Michaud, Ztschr. Physiol. Chem. **39**, 405 (1909).

<sup>15</sup> Ztschr. Physiol. Chem. **60**, 425 (1909).

Experiments are described in feeding zein and gelatin, two proteins which are "incomplete" chemically, in that they lack certain cleavage products known to be present in animal proteins. It is shown that the animal can utilize the nitrogen of zein very efficiently for repair of the losses due to endogenous or tissue metabolism. The average utilization of zein nitrogen for this purpose, was about 80 per cent, for gelatin 50 to 60 per cent. No evidence was obtained of the formation of additional body tissue from zein, even when the latter was fed in great excess over the maintenance needs of the animal.

Experiments in feeding casein as the only protein, resulted in increases of the body protein of 20 to 25 per cent. These are the most successful growing experiments yet reported in which but a single protein was fed.

The experimental data presented do not harmonize with the most widely accepted theories concerning the mechanism of protein metabolism. The repair processes are shown to be of a different character from the processes of growth. The results of the work here presented are believed to indicate that the processes of cellular catabolism and repair do not involve the destruction and resynthesis of an entire protein molecule.

## A METABOLISM CAGE FOR THE PIG

E. V. McCOLLUM and H. STEENBOCK

During the past four years in this laboratory, pigs have been in use continuously as experimental animals in nutrition studies. They have proven especially satisfactory in certain respects. Almost all of our exact knowledge of the chemistry of nutrition has been gained through studies conducted with carnivora and herbivora, in each of which types dietary habits and a number of well marked differences in metabolic processes depart widely from the human.

In the pig the plan of the nutritional processes is closely similar to that in man. In marked contrast to man, the period of infancy and therefore of growth are exceedingly short for the great size attained. The pig is probably endowed with a greater ability to utilize food for growth than any other animal large enough for satisfactory quantitative collections of the urine during early life when growth is most rapid. Added to this are its indolent habits which enable it to remain closely confined for long periods without chafing, and its almost unfailing appetite even for food of low palatability. It almost never regurgitates its food, and if freed from intestinal parasites is not subject to digestive disturbances.

One of us has noted elsewhere that a pig will take for long periods a liberal energy supply in the form of pure starch, scalded and made into a thin soup, with salt mixture supplying all necessary mineral elements. (See page 76). This animal offers, therefore, special advantages for the study of many problems connected with protein metabolism.

In the progress of our work a metabolism cage has been evolved suitable for the quantitative collection and separation of the excreta of the pig. Since this seems now to be perfected, we have thought it advisable to describe its construction and operation.

## CONSTRUCTION OF THE CAGE

The cage is shown in all essential details in the accompanying illustrations. It consists of a main cage in which the animal is confined except when being fed and a feeding stall which communicates with the main cage by the sliding doors *N* and *C*. The large retaining cage as shown to the right in Figure 1 consists of a square zinc lined box having a hinged

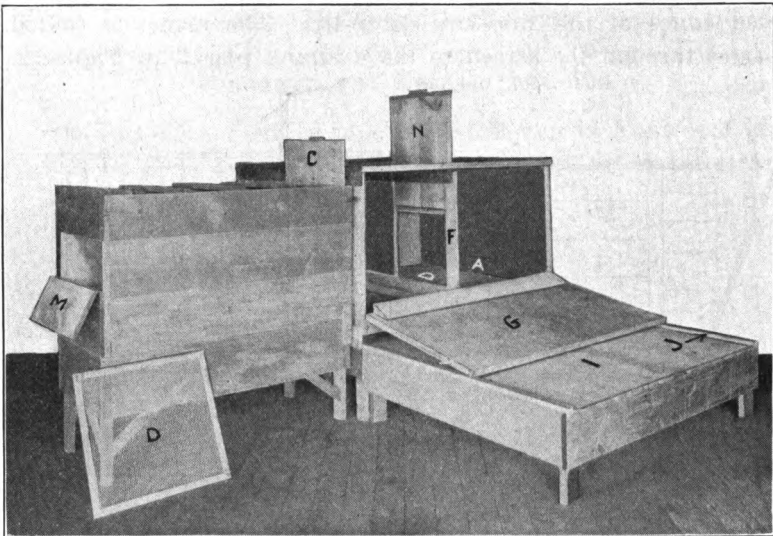


FIGURE 1. METABOLISM CAGE FOR PIG, RIGHT SIDE

side, *G*, a hinged iron barred top, a dividing partition *F*, and a screen floor *D*. The whole is supported on four iron castors which enable the cage to be rolled along a wooden track *J*, either over the zinc topped draining table or over the accessory table *I*, exposed in the picture. The walls of the cage are zinc covered to within 4 1-2 inches of the bottom of the cage at which place they are beveled inwardly 2 1-2 inches. The hinged side *G*, is also zinc covered and beveled as shown by *A*, like the other wall. This side serves as the door through which the animal can be introduced and solid excreta removed. The top of the cage consists of a grating formed of  $\frac{3}{8}$  inch iron bars held in a wooden 2x4 inch frame. From these bars, by means of hooks, the zinc covered dividing parti-

tion *F*, of the cage is suspended. This partition makes it possible to adjust the size of the cage for different sized animals and thus avoids the needless exposure of floor space.

The floor of the cage consists of a heavy screen four meshes to the inch of number 11 wire which is fastened by means of staples to a 2x4 inch frame and further supported by horizontal iron bars  $\frac{3}{8}$ x1 inch at 15 inch intervals. These bars are likewise inserted in the 2x4 inch frame which is introduced from the bottom of the cage up to within  $\frac{1}{2}$  inch of the beveled edges of the zinc covered walls. The urine as voided passes through the screen to the draining pan *B* in Figure 2,

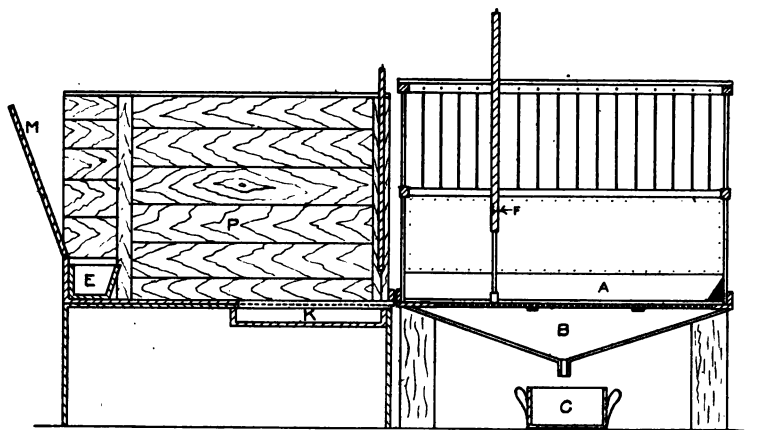


FIGURE 2. CROSS SECTION OF METABOLISM CAGE FOR PIG

which is zinc covered and drains to its center with a fall of two inches to the foot dropping into receptacle *C*. The space of  $\frac{1}{2}$  inch between the beveled lower edges of the zinc covered wall, and the screen floor serves a double purpose. In the first place it obviates the presence of angles into which feces might become lodged and from which they could be removed with difficulty. With this construction all feces can be readily removed from the screen floor. The periphery of the screen floor is never in contact with feces, and this portion is easily gotten at for brushing and cleaning by turning the cage on its side so that the bottom is accessible. In the second place it permits the superimposition of a second lighter screen over the screen floor and below the beveled edges of the walls. The screen is shown at *D* in Figure 1. Between these screens is placed a single thickness of cheese cloth stretched over the upper

screen by perforating the corners of the cloth by the corners of the screen.

This last device has been employed only when rations rich in wheat bran were used. Such rations produce bulky and granular feces which readily fall through the screen floor upon the draining pan, the slope of which facilitates distribution and contamination with urine. In all ordinary work this device has not been used except as an easy means of keeping the cage clean when no collections were being made while a pig was becoming familiar with the cage.

#### FACILITIES FOR FEEDING THE PIG

The auxiliary feeding cage is best shown in Figures 3. and 4. It consists essentially of a narrow box fitted by means

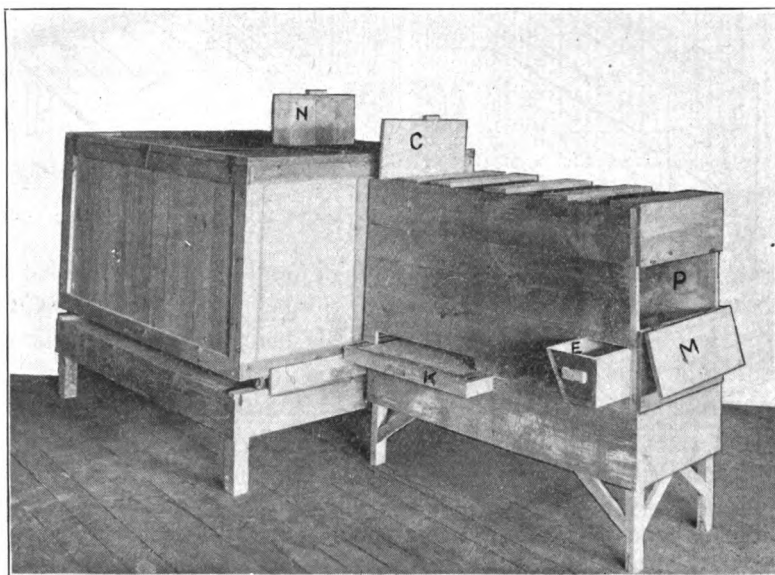


FIGURE 3. METABOLISM CAGE FOR PIG, LEFT SIDE

of cleats on the inside to the supporting bench. The latter is fitted in the rear with a sliding zinc lined drawer which passes under a heavy screen *D*, which makes up the rear part of the floor in the cage. This screen is of the same construction as the one previously described in connection with the main cage. This screen and the sliding drawer beneath serve as a



safety device in case the pig should void any urine when in the feeding stall. In practice this almost never happens if the pig is left in the stall only while busy eating.

The feeding cage proper consists of a narrow box fitted with a sloping floor and beveled tin lined walls so as to drain into the drawer below. The rear trap door, *C*, communicates with the large cage while through the front hinged door *M*, the animal can be fed in the galvanized-iron-lined trough *E*. As required, the trough may be slid sideways and removed from

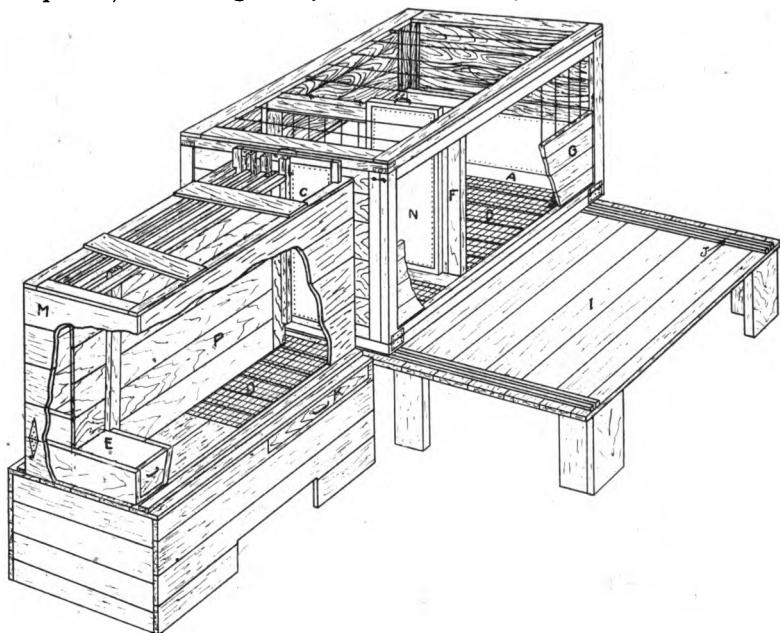


FIGURE 4. DETAILED VIEW OF METABOLISM CAGE FOR PIG

the cage for cleaning. To prevent the animal from scattering his feed by turning around while feeding, the cage is provided with a movable partition *P*, which fits in between cleats and thus can be adjusted to confine an animal as closely as desired. The part of the floor on which the pig's fore feet stand, *H*, in Figure 5 is zinc lined to facilitate cleaning.

The special difficulties connected with the feeding of a pig kept in a metabolism cage are obviated by transferring the animal to the feeding stall at meal time. Advantage should be taken of the absence of the pig from the main cage, for cleaning the latter at this time. The sliding doors *N* and *C*

are lifted and the pig will of his own accord after a brief period, hasten to the trough *E* in which the ration has been placed. The doors are allowed to drop and at once the hinged side *G* is lowered to give access to the cage. The feces are removed so far as possible by means of a broad steel spatula and the remainder are brushed through upon the draining pan by means of a strong steel brush. The cage is then pushed along the track *J*, so that the draining pan can be carefully brushed free from feces, the latter being collected in a pan. Previous to washing the screen floor of the cage it is our custom to tip the cage upon its side and thoroughly brush the bottom with the steel brush. When the pan is free from feces the cage and pan are washed with the usual precautions in such work.

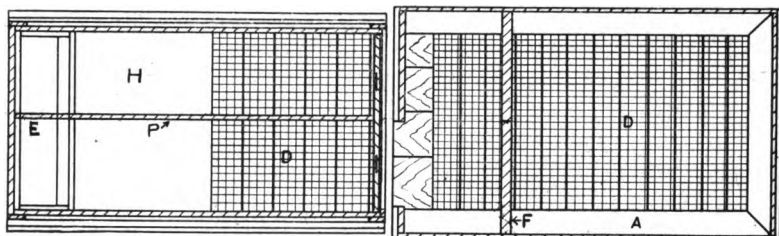


FIGURE 5. FLOOR PLAN OF METABOLISM CAGE FOR PIG

This can usually be done in the time during which the pig will busy himself with cleaning up the trough. He is transferred again to the cage when all is ready.

We have found it of advantage, to use only male pigs for cage work since with these the urine is deposited within a narrow radius near the center of the floor and, the cage being adjusted to just permit the animal to turn around and to lie stretched out when placed diagonally, the feces will always tend to be deposited near the walls.

Another important matter is that of clipping the hair from the head of the animals. This prevents food from adhering to the face. It is a simple matter to wipe any adhering particles from the nose and face with a small cloth before replacing in the main cage after eating.

In work with pigs confined in the cage we have found it advantageous to modify the character of the feces by giving either agar-agar or calcium phosphate or both. By this

means the successful operation of the cage without contaminating the urine is rendered much more easy and certain.

The successful operation of the cage requires visiting it several times a day so as not to allow the accumulation of feces on the floor for any great length of time. With care, however it gives good results.





# Metabolic Water: Its Production and Role in Vital Phenomena

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S. M. BABCOCK

Water is essential to life and during the period of development it is the most abundant constituent of living organisms, its amount ranging from about 40 to nearly 100 per cent of the total weight. Some of this water is imbibed directly, some of it is taken with the solid food which is rarely dry, and some of it is formed within the organism by metabolic changes in the organic constituents of the food and tissues, induced by respiration and other vital processes. The relative amount of water derived from each of these sources depends upon the kind of organism, its period of growth, the nature of its food, its environment, and its activities.

The chief functions of water are to dissolve nutrients and serve as a medium for their distribution, to remove injurious waste products from the cells, to control the temperature, within narrow limits, by its evaporation, and in chlorophyl producing plants to supply material for the synthesis of organic matter. In general, plants and most animals require an abundant and frequent supply of water from external sources at all periods of growth in order that these functions may be properly performed. There are, however, particular stages in the life history of both plants and animals in which metabolic water is sufficient for all purposes, for considerable periods of time. Thus in the resting periods of deciduous plants, in bulbs, in tubers, and especially in seeds and spores ample water for all vital processes is provided by the slow oxidation that takes place as a result of direct respiration. This is also true in the case of hibernating animals that receive no water from external

sources for several months, although water is constantly lost through respiration and the various excretions. In addition, many varieties of insects such as the clothes moths, the grain weevils, the dry wood borers, etc. are capable of subsisting, during all stages of development, upon air dried food materials containing less than ten per cent of water; in these cases, nearly all of the water required is metabolic.

While the production of metabolic water has been recognized by all students of plant and animal physiology, there has heretofore been no distinction made between its functions and those of imbibed water. It has evidently been assumed, because the molecular structure of water from various sources is the same, that it always serves the same purposes in vital processes, whether derived from external or internal sources.

It is the purpose of this paper to show that metabolic water is not only produced in considerable quantity from the organic constituents of the food and tissues of plants and animals by oxidation and by dehydrating reactions, but also that water so produced performs a different function from imbibed water and in many instances, if not in all, is essential to the growth and continued life of the organism.

#### SOURCES OF METABOLIC WATER

The most obvious source of metabolic water is the oxidation of organic matter comprising the food and tissues of an organism, by means of free oxygen derived from the air during respiration. This production of water is always associated with an absorption of free oxygen and an evolution of carbon dioxide, the carbon dioxide being of practically the same volume as the absorbed oxygen.

Many organisms also, when deprived of free oxygen, are capable of maintaining, for a short time, certain of the respiratory functions, and deriving energy from food material and from tissues by breaking up the molecular structure into new forms of a lower order. This is known as intramolecular respiration and like direct respiration, results in the production of both water and carbon dioxide.

Metabolic water is also produced by all organisms through changes in the molecular structure of substances composing its nutrients or its tissues. The transformation of dextrose or in-

vert sugar into cellulose, starch, or cane sugar, and the formation of muscular fiber, and other complex proteids from peptones or from amino acids, are examples of such reactions. No carbon dioxide is evolved in changes of this nature. This source of metabolic water is probably the most important of all, if aggregate quantities are alone considered, since the same carbon nucleus may function alternately in hydrolytic and dehydrating reactions an indefinite number of times.

*Respiration*, either direct or intramolecular with its consequent loss of organic matter and evolution of carbon dioxide continually takes place in living organisms of all kinds and its total suspension, even for a brief period, is the best evidence of death. This becomes more apparent when it is realized that the energy required for maintaining vital activity of all kinds is, in its final analysis, wholly derived from the slow combustion of organic substances, stored within the organism.

The phenomena of photosynthesis, which enables green plants to utilize solar energy for the accumulation of food materials from which tissue is formed, are not opposed to this view since the further use of such substances by a plant requires a considerable expenditure of energy which can be derived only from oxidation or other degradation of substances already formed. The function of these stored materials in promoting the vital activity of a plant is entirely analogous to that of food materials supplied to the digestive tract of an animal, which serve no useful purpose for the animal until they are digested and assimilated. Photosynthesis is a means of collecting food material which, when assimilated and incorporated into the cellular structure of a plant, may be oxidized and a portion of its stored energy utilized for maintaining the vital processes. Until these materials become a part of the cellular structure, or of the circulatory fluids, they can contribute no energy to the support of an organism. Solar energy converts compounds, which the plant is not able to use directly, into raw food materials. Assimilation of this raw material is only accomplished by destruction of material previously assimilated. These changes are all, directly or indirectly, dependent upon energy derived from combination of organic nutrients with oxygen acquired by respiration.

*Water Derived from Oxidation of Nutrients and Tissue* The substances oxidized by both plants and animals, to supply vital



energy, consist chiefly of carbohydrates, fats, and proteins. All of these substances contain hydrogen, and their complete oxidation produces a quantity of water equal to nine times the weight of hydrogen present in the original substance. Thus one hundred parts of cellulose or starch,  $(C_6H_{10}O_5)_n$ , containing 6.17 per cent of hydrogen, gives 55.5 parts of water; one hundred parts of anhydrous dextrose containing 6.66 per cent of hydrogen gives 60 parts of water etc. Most of the fats yield more than their weight of water, while proteins, when completely oxidized, give from 60 to 65 per cent of water. Protein metabolism, however, is not a factor that affects the total water content of a plant, since in general, the nitrogenous substances resulting from respiration are again assimilated, as much water being absorbed in their reconstruction as was set free in their breaking down. On the other hand, protein metabolism may be an important factor in the transfer and distribution of water from place to place, since the destructive and synthetic reactions often occur in cells quite remote from each other. Animals, however, are unable to utilize the final products of protein metabolism which are in most cases poisonous and must be removed from the tissues by excretion in various forms, the principal of which are urea, uric acid, and ammonia. The amount of metabolic water derived from the breaking down of protein, when urea is excreted, is about 42 per cent of the weight of protein, when uric acid is excreted nearly 53 per cent, and when ammonia is excreted about 32 per cent.

The amount of metabolic water formed by oxidation during any period is proportional to the rate of respiration. Every circumstance which hastens or retards respiration also affects, in the same way, the production of metabolic water. The rate of respiration differs with the type of organism, even though external conditions may be the same. As a rule it is slower with plants than with animals, and slower with cold blooded than with warm blooded animals. It varies widely for the same individual at different stages of development and even in different organs of the same organism at the same time. In dry seeds and spores respiration is practically suspended, it being possible to detect it only by observations extending over long periods of time; it is also slow in bulbs and tubers, and in the whole plant during the winter; it is most pronounced when vital processes

are most active, as during the germination of seeds, at flowering, and at other periods of rapid growth.

*Intracellular Production of Metabolic Water* . Respiration is a function peculiar to active protoplasm. Since this substance which constitutes the physical basis of all life is, with minor exceptions, organized as distinct cellular units, it follows that the production of metabolic water is mostly confined to the interior of such cells.

In consequence of this local production of water, the concentration of the cell contents is reduced, both by the production of water and by the elimination of soluble organic matter. This change in concentration disturbs the osmotic equilibrium between the fluids within and without the cell and a movement of nutrients by osmosis is induced towards the depleted centers. It is not an essential condition for these effects that soluble nutrients within a cell be completely oxidized; it is sufficient if the molecular structure of nutrients within a cell be changed, especially if the change results in the liberation of water, or if the nutrients be rendered insoluble, as occurs when soluble carbohydrates are deposited in the form of starch, cellulose, or fat, or when soluble and diffusible nitrogen compounds are converted into insoluble tissue or even into non-diffusible proteins.

Water from an external source does not serve these purposes, since its immediate effect is to reduce the concentration of soluble nutrients in the circulatory fluids to a point below that in the fluids of the cells and thus cause a movement of nutrients away from, rather than towards the points where they are most needed. If the distribution of nutrients is alone considered, absorbed water tends towards the starvation of the cells. Absorbed water does, however, facilitate the removal of waste products, replaces water lost through evaporation and, in the case of chlorophyl producing plants, supplies material for the synthesis of organic nutrients and, for this reason, an abundant supply of water from external sources is absolutely essential for the growth of this class of plants. With parasitic plants, and with animals, which derive all of their organic nutrients from chlorophyl producing plants, imbibed water is not so essential to life; with these the chief function of imbibed water is to aid in the removal of waste products, the metabolic water being in most cases sufficient for transferring nutrients and for replacing the ordinary losses incurred by respiration and evaporation.

METABOLIC WATER IN SEEDS<sup>1</sup>

A seed consists of an embryo plant in a nearly dormant condition, surrounded by sufficient reserve food material to nourish it until it has reached a stage of development in which it is capable of assimilating carbon dioxide from the air, and inorganic substances from the soil.

Although the embryo of an air dried seed is generally assumed to be entirely dormant, it continues to perform certain vital functions similar, except in degree, to those of a mature plant. Chief among these is respiration which is manifested by an absorption of oxygen, an evolution of carbon dioxide, the production of water, and a consequent loss of dry matter.

*Water Content of Seeds* Since the production of water from the oxidation of organic matter is a necessary consequence of respiration, all viable seeds must contain some free water at all times. The water content of air dried seeds of most economic plants, ranges from about five to more than twenty per cent, depending upon the degree of saturation of the surrounding air. For most of the common grains it is about ten per cent.

It is impracticable to remove all water from seeds except by prolonged exposure to a temperature approximating 100° C. Seeds dried in this way and again exposed to ordinary air, even though it be moderately dry, absorb from five to ten per cent of moisture in a short time. Seeds kept in a saturated atmosphere for three or four weeks usually contain over 30 per cent of water and if temperature conditions are favorable such seeds will germinate without having been in direct contact with water or with a moist surface. Between these limits seeds may contain any proportion of water depending upon the degree of saturation of the air and the time of exposure. The strong affinity of seeds for water and the persistence with which the absorbed water is retained at ordinary temperatures indicate that there is a feeble molecular combination of water with substances comprising the seed analogous to that occurring in crystals containing water of crystallization. There is also little doubt that the physical structure of a seed, especially of its outer covering

<sup>1</sup> Unless otherwise stated, the observations upon seeds recorded in this paper, refer to corn, (*Zea mays*); it is believed, however, that they apply to other varieties of seeds as well. Corn has been selected for the tests because of the large seed and the ease with which the embryo may be separated from other parts of the germinating kernel.

or hull, is an important factor affecting both the absorption and retention of water. The influence which the hull exerts upon the retention of water is illustrated by Table I, in which the times required for drying whole kernels of corn and kernels from the same ear, with the hull cut through in a number of places with a sharp knife, and other kernels divided into particles of different size, are compared.

The same number of kernels and approximately the same weight of material was dried in each case in a steam oven the temperature of which was approximately 97° C. It should be borne in mind that at this temperature drying proceeds at a considerably lower rate than at 100° C. Fine corn meal dries to practically constant weight in 10 to 12 hours, at this temperature.

TABLE I. INFLUENCE OF HULL ON ESCAPE OF MOISTURE

 Kernels of corn (*Zea mays*) were dried at 97° C.

Hours exposed	PERCENTAGE LOSS IN WEIGHT			
	Whole kernels	Kernels with broken hulls	Split kernels	Meal
5 .....	4.47	6.04	6.20	7.94
24 .....	6.85	8.04	7.95	8.89
30 .....	7.07	8.22	8.12	8.97
48 .....	7.68	8.54	8.52	.....
72 .....	8.18	8.77	8.68	.....
96 .....	8.47	8.95	8.90	.....
120 .....	8.76	.....	.....	.....
144 .....	8.89	.....	.....	.....
168 .....	9.01	.....	.....	.....

It appears from the table that split kernels and those with only the hulls cut through, dry at practically the same rate, but this is considerably slower than with rather coarse meal. The difference in the rates of drying of these kernels and of the meal, is best explained by the relatively larger surface exposed in the particles of meal. On the other hand, the kernels with unbroken hulls have approximately the same surface as the others but require a materially longer time to lose the same weight, at 97° C. This difference must be attributed to the protecting influence of the hull. Similar results have been obtained in a great number of tests in which whole kernels of corn have been dried at 97° C. In practically all such trials not less

than 168 hours have been necessary to reduce the loss in weight to less than .1 per cent in twenty-four hours, whereas meal or broken kernels, containing as much moisture, have dried to the same extent in about twenty-four hours.

The persistence with which whole kernels of corn retain moisture is still better illustrated in an experiment designed to show the effects of respiration and the production of metabolic water in air dried corn. In this experiment, corn was placed in a desiccator over sulphuric acid and the loss of weight determined after varying intervals. Samples from the same lot were dried in a steam oven, to practically constant weight, at the beginning of the test, and afterwards, corn from the desiccator, was dried in the same manner at dates, when observations for weight were made. The percentages given in Table II are all calculated up-

TABLE II. INFLUENCE OF DRY AIR ON LOSS OF MOISTURE

Loss of weight of corn exposed to a temperature of 97°C. compared with that of corn kept in a desiccator over sulphuric acid.

Dates Weighed	Days of Test	PER CENT LOSS IN WEIGHT		
		Over H <sub>2</sub> SO <sub>4</sub>	At 97°C.	Total
1908.				
May 9.....	0		8.42	8.42
Aug. 21.....	104	6.15		
Oct. 15.....	159	6.64		
1909.				
Feb. 20.....	287	7.32	1.27	8.59
May 4.....	369	7.48	1.20	8.68
Sept. 28.....	507	7.92	0.97	8.89
1910.				
Feb. 21.....	653	8.13	0.90	9.03
May 13.....	734	8.25	0.84	9.09
Sept. 8.....	852	8.36	0.74	9.10
1911.				
Feb. 7.....	1004	8.53	0.71	9.24
May 9.....	1095	8.56	0.39	8.95
Sept. 18.....	1227	8.69	0.00	8.69

on the weight of the air dried sample at the beginning of the test. The corn used was a white dent variety of the crop of 1907; it was well cured as shown by a number of germination tests including more than 100 kernels in which every kernel germinated with strong healthy sprouts; it was placed in the desiccator May 9, 1908.

If it be assumed that the loss in weight at 97° C. represents water only, and that the organic matter of the seed is practically non-volatile, at the room temperatures in which the desicca-

tor containing the corn was kept, there is still considerable water remaining in this corn at the end of  $3\frac{1}{2}$  years. In any case there has been a gradual and continual loss of dry organic matter which on February 7, 1911 amounted to .82 per cent of the original weight of the corn. A slow evolution of carbon dioxide, throughout the whole period, indicates that this loss is the result of oxidation and it is believed that the oxidation is incident to respiration of the living cells of the embryo. If the loss is due to oxidation, about 60 per cent of it, or nearly .5 per cent of the corn at the beginning of the test is water, some of which is still present in the grain. It is believed that this constant production of water, small as it is, is essential for maintaining the vitality of the embryo, and that it will continue to be formed so long as the seeds are viable.

The total metabolic water produced in the corn during the  $3\frac{1}{2}$  years exposure to dry air is larger than is indicated by the preceding results, since the oil contained in corn is a semi-drying oil which absorbs oxygen directly from the air without liberating an equivalent weight of carbon dioxide. In consequence of this, the dry matter of the grain is increased by an amount equal to the oxygen absorbed and a correction equal to this should be added to the apparent loss of water. This reaction takes place slowly at ordinary temperatures, but quite rapidly when the temperature is raised.

A sample of commercial corn oil was dried by contact with anhydrous calcium chloride and afterwards exposed, in a thin layer, to air at a temperature of approximately  $97^{\circ}$ . During the first twenty-four hours, this oil gained 4.58 per cent in weight, and after forty-eight hours the gain was 4.63 per cent. No further increase was noted after longer exposure. The oxidized oil was much less soluble in ether than the fresh oil. The oil used in this test had been in the laboratory several years and doubtless had already absorbed some oxygen. It is probable that fresh oil, under similar conditions, would absorb at least 5 per cent of its weight of oxygen.

The ether extract from fresh corn of the variety placed in the desiccator, averages about 5 per cent; that from the desiccated corn was only 3.82 per cent, indicating a considerable oxidation of the fats. No doubt these fats are nearly saturated with oxygen and the dry organic matter of this corn has been increased

from this cause by as much as .25 per cent, all of which should be added to the amount of metabolic water as indicated by the data. The decrease in total water, when the last determinations were made, may have been caused by this change.

*Influence of Drying Upon Germination* In a number of tests, including more than 100 seeds from the same lot of corn, made at the beginning of the experiment, all kernels germinated with strong healthy sprouts; subsequent tests of the seeds exposed to air dried by sulphuric acid, made at each date when the corn was weighed, showed a gradual weakening of the sprouts although all germinated until September 28, 1909, when only seventeen out of twenty seeds germinated; February 21, 1910, sixteen out of twenty germinated; May 13, 1910, sixteen out of twenty germinated; September 8, 1910, fourteen out of twenty germinated; May 9, 1911, ten out of twenty germi-

TABLE III. GERMINATION OF DESICCATED AND AIR DRIED CORN

Germination of corn kept three years in a desiccator over sulphuric acid, compared with that of corn from the same lot kept in a cloth sack exposed to ordinary air. Twenty kernels were tested in each case.

Days in Germinator	DESICCATED CORN		CORN FROM SACK	
	Tested be- tween wet filters	Immersed in hydrogen peroxide	Tested be- tween wet filters	Immersed in hydrogen peroxide
1.....	0	0	0	0
2.....	4	0	12	8
3.....	8	0	18	18
4.....	10	0	20	20
6.....	10	0		
Corn from same lots four months later				
1.....	0	0	0	0
2.....	0	0	12	10
3.....	0	0	20	20
4.....	5	0		
5.....	7	0		
6.....	9	0		

nated; and September 18, 1911, nine seeds out of twenty germinated. At the last date, seeds from the same lot which had been kept in a cloth sack freely exposed to air that was not excessively dry, all germinated in a normal manner with strong sprouts. The sprouts upon the desiccated seeds were all feeble. The weakened vitality of the desiccated seeds, compared with those kept in the cloth sack, is more clearly shown in Table III in which is given the time required for the germination of both samples, when placed between moist filters, and when immersed in hydrogen peroxide. Data are given for the last two tests.

In all cases the sprouts upon the desiccated corn were very weak and were soon killed by the growth of molds.

Nearly all of the metabolic water formed during this period must have been set free in the embryo, since the protoplasm, which functions in respiration, is chiefly located in this portion of a seed. As perfectly dry protoplasm is incapable of respiration, it seems highly probable that even the small amount of water formed under conditions of extreme dryness may serve an important purpose in prolonging the vitality of seeds.

The rate of respiration of seeds is, within certain limits, determined by the water content; with less than 10 per cent of water it proceeds very slowly and in this condition of dryness most seeds remain alive and capable of growth for long periods with a very limited amount of air. With 15 to 20 per cent of water, respiration is quite active so that oxygen is soon exhausted from a closed vessel filled with seeds, and direct respiration is suspended. If this condition is continued for an extended period, death of the seed ensues.

It is a well established fact that seeds do not remain viable for a long time when stored in large quantities in tight bins. This is especially true of recently harvested corn, wheat and oats. These grains often contain, at this time, more than 20 per cent of water which condition stimulates respiration to a point where the seeds become warm, damp, and finally musty. The rise in temperature is due chiefly to respiration, the dampness to the production of metabolic water, the musty condition to the growth of molds and other fungi, the development of which is favored by warmth and excessive moisture.

Frequent exposure to air, by moving from one bin to another, serves to maintain a better condition in all grains when stored in bulk and this plan is resorted to in all store houses. The usual practice with corn is to store it, on the cob, in well ventilated cribs, so that air may circulate freely throughout the whole mass. Corn intended for seed should never be stored in bulk in large quantities, even on the ear; the safest plan is to reduce the water content to, at most, 10 per cent, by artificial heat, after which the ears should be kept in small piles through which moderately dry air may circulate freely; it should not be stored in a damp place, nor should it be shelled long before planting time. Seed grains are more reliable if kept in medium sized



cloth sacks so placed that air may circulate freely between them. In this way normal respiration of the seeds is assured, excess of moisture is removed, and vital activity greatly prolonged.

When grain is stored in large quantities, its moisture increases slightly, especially if the conditions are unfavorable to evaporation. The gain that occurs in these cases has always been attributed wholly to water absorbed from nearly saturated air. This factor is no doubt operative but it is probable that metabolic water formed by the respiration of the seeds, coupled with a reduced evaporation, has a marked influence upon the increase in the water content of the grain.

It must constantly be borne in mind that seeds as well as growing plants and animals must at all times, have a supply of free oxygen if the highest state of vitality is to be maintained, although the amount required by air dried seeds is quite small. Even when seeds are so dry that the growth of molds and bacteria is inhibited, respiration still persists and if the seeds are kept in air tight vessels, moisture due to production of metabolic water accumulates, the free oxygen is replaced by carbon dioxide, and after a time the seeds die.

*Influence of Carbon Dioxide upon the Viability of Seeds* It is probable that seeds, as well as growing plants, may for a short time derive the energy required for maintaining vital processes through intramolecular respiration, but a total suspension of direct respiration always results, sooner or later, in the death of a seed. The length of time which a seed remains viable, when oxygen is excluded, depends upon the rate of respiration, which varies with the variety of seed, its water content, and the temperature to which it is exposed. Air-dried corn, containing less than 10 per cent of water, respire very slowly, even at summer temperatures, and at temperatures below freezing its respiration can scarcely be detected; such corn can be kept, for a year or more, in a closed vessel without materially reducing its germinating power. Germinating corn, containing about 40 per cent of water, dies very quickly when oxygen is withheld, and corn containing more than 20 per cent of water loses its germinating power in less than two months, if kept in an atmosphere of carbon dioxide. At temperatures below freezing the effect is much slower, because respiration is greatly re-

duced. This principle is illustrated in the following tests made with corn containing various amounts of water, from which the oxygen was excluded by filling the containing vessel with carbon dioxide.

Seeds from a well cured ear of yellow dent corn, of the crop of 1909, containing about 6 per cent of water, every kernel of which germinated in several tests, were hermetically sealed in an atmosphere of carbon dioxide, November 20, 1909. The vessel containing these seeds was kept in a drawer, in the laboratory, at a temperature averaging about 20°C., (68°F.), and was not opened until November 11, 1910. At this time considerable pressure had developed in the vessel, showing that intramolecular respiration or anaerobic fermentation had taken place. The appearance of the corn had not changed. Comparative germination tests were made of this corn and of corn from the same ear that had been kept in air. These trials were made by placing ten kernels from each lot between folds of moist filter paper, and also by immersing the same number of seeds in water containing 1½ per cent of hydrogen peroxide. The results are given in Table IV.

TABLE IV. EFFECT OF CARBON DIOXIDE UPON VIABILITY OF SEEDS  
Germination of corn kept 365 days in air compared with that of corn kept in carbon dioxide.

Hours required for germination	Corn kept in air, Kernels germinated		Corn kept in carbon dioxide, Kernels germinated	
	In wet filters	In H <sub>2</sub> O <sub>2</sub>	In wet filters	In H <sub>2</sub> O <sub>2</sub>
24.....	0	0	0	0
48.....	8	3	0	0
72.....	10	10	7	9
96.....			10	10

Although all kernels from both lots finally germinated, it is evident, from the longer time required for those kept in carbon dioxide, that their germinating power had been considerably weakened by exclusion of oxygen.

Another sample of yellow dent corn, crop of 1910, taken soon after it was husked, was placed in carbon dioxide as in the first case, November 15, 1910. This corn contained 29.66 per cent of water, at the beginning, and in germinating tests made at this time, 80 per cent germinated in contact with wet filter

paper, and 90 per cent germinated when immersed in hydrogen peroxide: During the first twenty-four hours, there was a marked decrease in the pressure of gas within the vessel indicating an absorption of carbon dioxide by the corn; after this time the pressure increased quite rapidly because of carbon dioxide liberated by intramolecular respiration and during the next thirty days considerable gas was evolved. From this time on the pressure remained practically constant, indicating that respiration had ceased and that the seeds were dead. On January 12, 1911, the corn was examined and germination tests made. At this time the corn was bright and apparently sound, but had a peculiar acid odor similar to corn silage. The water content had increased from 29.66 per cent to 33.94 per cent, giving unmistakable evidence of active intramolecular respiration or of anaerobic fermentation. None of the grain germinated, either in contact with wet filters or in solution of hydrogen peroxide. Kernels from the same ear kept in air, all germinated by both methods, a better result than was obtained at the beginning with the uncured corn.

A sample of white dent corn, crop of 1910, containing 20.07 per cent of water was placed in an atmosphere of carbon dioxide November 16, 1910. This corn behaved in a similar manner to that in the previous test, but the acid odor, after an exposure of two months, was not quite so pronounced. None of this corn germinated, while of the kernels kept in air every one germinated both in contact with wet filters and in a solution of hydrogen peroxide.

Another sample of white dent corn, crop of 1909, that had been kept in the laboratory exposed to air one year, and that contained 8.7 per cent of water, was placed in carbon dioxide November 17, 1910. These seeds were removed and tested July 26, 1911, after an exposure to carbon dioxide for a little more than eight months. The seeds were found to be in good condition and the ten tested, germinated, both in contact with wet filters and in a solution of hydrogen peroxide. In this respect the seed was fully as good as seed from the same ear that had been exposed to air.

During all of the above tests with carbon dioxide, direct respiration must have been suspended, except so far as the small amount of oxygen retained in the tissues of the seed may have

served for the purpose. The seed must, therefore, have derived practically all of the energy required for maintaining its vital functions, through intramolecular respiration. Some of the products resulting from intramolecular respiration are injurious to living cells when they are present in even moderate amounts, but these products are formed so slowly when the water content is below 10 per cent that no injurious effects are evident for a long time; finally, however, the accumulation of these products is sufficiently large to cause the death of a seed, no matter how low its water content may be. With a water content of 20 per cent or over, intramolecular respiration is so rapid especially if the temperature be favorable, that death of a seed results in a short time.

*Injury to Seed through Storage in Bulk* When grains are stored in bulk, the heat generated by the slow respiration that occurs is not readily dissipated and the temperature gradually rises; with the increased temperature, respiration, both direct and intramolecular, is augmented and in a short time oxygen is practically excluded, so that only intramolecular respiration is possible. Unless grain is quite dry, (it should not contain more than 10 per cent of water), storage in bulk without frequent aeration, to reduce temperature and to remove the accumulated carbon dioxide and water, is sure to result in damage. Naturally, greater injury occurs, under such conditions, near the top of a bin where some oxygen enters by diffusion, since this permits some direct respiration of the grain to take place, and also allows molds and other destructive organisms to develop; both of which contribute to a more rapid rise in temperature.

Experiments made with large quantities of corn by J. W. T. Duvel<sup>2</sup> to determine the deterioration of corn in storage, and later, in cooperation with Laurel Duval<sup>3</sup> to determine the shrinkage of corn in storage, confirm the above results and conclusions in every respect.

In the later test, 28,000 pounds of shelled corn with an average water content of 18.8 per cent and an average germination of 89.6 per cent were placed in the wooden hopper of a 30,000 pound elevator scale and the weights and temperatures observ-

<sup>2</sup> Cir. 43—Bur. Plant Industry. U. S. Dept. of Agr.

<sup>3</sup> Cir. 81—Bur. Plant Industry. U. S. Dept. of Agr.

ed at intervals. The experiment was begun January 5, 1910, at which time the average temperature of the corn was 20°F. Fifty days later, February 24, the total loss in weight was 30 pounds. April 8, the total loss amounted to 60 pounds, a little more than .2 per cent of the initial weight, and the average temperature was 46.3°F. April 21 the total loss was 107.5 pounds and the average temperature was 69.5°F. The highest temperature at any point in the corn at this time was 87°F., near the top where it was exposed to air, and the lowest temperature was 51°F. near the bottom where it was protected from air by the corn above. Up to this time the corn was in good condition, but after this there was a marked increase in temperature and a decided falling off in quality. The maximum temperature May 2 was 138°F., near the top, May 12 133°F. and May 14, at the same point, 119°F.

The high maximum temperature must be attributed chiefly to the accumulation of heat generated by direct respiration of the cells of the embryos, although fungus organisms undoubtedly contributed something towards it. The decrease in temperature in the last few days, appears to be due to the death of the cells of the grain, and a consequent reduction in the rate of direct respiration of the corn, since there is no reason for believing that the growth of fungi would diminish, so long as an abundance of suitable nutrients was available. The active cells of the grain are killed by the exclusion of oxygen and the high temperature, rather than by direct attack of lower organisms, since living cells are extremely resistant to such organisms as occur in grain. The relatively low temperature at the bottom of the bin is due to a practical suspension of direct respiration of the corn and to the inability of other organisms to develop in the absence of oxygen.

The effects of respiration are also shown by the loss of weight during the test. So long as the temperature remained near the freezing point the rate of respiration was very low and the loss in weight insignificant, being less than .4 per cent for the first 106 days. With the temperature above 50°F., however, respiration was enormously increased, causing a sharp rise in temperature, since radiation was necessarily slow from the large mass of grain.

May 14, the corn was run out of the hopper and elevated

three times thus giving it a thorough aeration and reducing the average temperature to 55°F., practically the same as the air. The exposure to air, and the mixing of the live with the dead kernels started active respiration again throughout the whole mass and by June 1, when the experiment was terminated, the corn was again hot. The total loss in weight, during the 147 days of storage, including the loss incurred when the grain was aerated, was 1970 pounds, a little more than 7 per cent. The average water content at the end was 14.7 per cent and the average germination was 1 per cent.

There were at the beginning of the test, 22,736 pounds of dry matter and at the end 22,204 pounds, a loss of 532 pounds. There was a direct loss of 1438 pounds of water, or over 5.1 per cent, of the initial weight, of the corn. In addition to this there must also have been lost an amount of water equivalent to the metabolic water arising from the total oxidation of the dry organic matter that had disappeared. Since sugars and fats contained in the embryo of seeds are the first substances to be oxidized in respiration, and since the total oxidation of 100 parts of these substances results in the production of from 60 to more than 100 parts of water, it is safe to assume that at least 60 per cent of the dry matter lost in these tests or 319.2 pounds has reappeared as water. This amounts to a little more than 1.1 per cent of the total initial weight. The "sour" condition and decreased viability of the corn at the end of the test are sure indications that intramolecular respiration had taken place or that anaerobic organisms were present in large numbers, since an acid condition does not result from the direct respiration of seeds.

*Enzymes of Seeds* Nearly all of the stored food materials of seeds, (starch, proteins, fats, etc.), are insoluble and unavailable for nourishing the embryo, until acted upon by certain specific ferments, (diastase, proteolytic ferments, lipase, etc.), which change them into soluble and diffusible products. These enzymes appear to be wholly absent from immature seed<sup>4</sup>. If they were present at this stage, even in small amount, all of the reserve food would be changed into a soluble form and either lost to the seed by diffusion to other parts of the plant,

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<sup>4</sup> See Experiment described in paragraph devoted to germination of immature seed on page 129.

or cause the seed to germinate prematurely, since moisture and temperature conditions are favorable for these purposes at the time when such substances are deposited. So long as a seed is attached to the parent plant by living tissue and receives its supply of food material therefrom, its condition is analogous to that of the fetus of an animal which receives its nutrients and respire indirectly through the parent. During this period there is no need for the action of such enzymes, and indeed their presence would be positively detrimental because the reserve food material, upon which the support of the future plant depends for its early development, would be dissipated.

As a seed matures, direct connection with the vascular system of the mother plant is broken and the seed soon dries to a point where the action of ferments is practically suspended. At this stage, direct respiration, which has previously been prevented by the succulent tissues, (either husks or fleshy fruits, which surround the seed) is also established. The rate of respiration is at first very slow for the reason that there is but little easily oxidizable material present in the embryo and also because free oxygen is still to a considerable degree excluded by the envelopes that surround the seed.

The protoplasmic activity of a seed is greatly stimulated by direct respiration and results in the production of small quantities of the specific enzymes, (diastase etc.), required to render the reserve food material available for the embryo. If the seed is moderately dry, containing less than 10 per cent of water, or if the temperature is near the freezing point or below, these changes are very slow, the seed remaining practically dormant for a long period, but capable of resuming its activity when favorable conditions are supplied. With a moisture content of over 30 per cent, and a favorable temperature, the rate of respiration is enormously increased and as a consequence diastase and proteolytic ferments are produced in sufficient quantity to rapidly convert the stored starch and proteins into soluble and diffusible forms. The abundance of easily assimilated food thus supplied stimulates the embryo, to still greater activity in this direction, and finally causes the seed to germinate. This principle is utilized in the manufacture of malt from barley grains which contain very little diastase until germinated under proper conditions, but afterwards are rich in this enzyme, while the starch, at first present, has wholly disappeared.

The embryo of a seed will starve and its respiration cease if suitable food is withheld for an extended period, the length of which varies with the variety of seed and its environment. This sometimes happens in old dry seeds in which the inverting enzyme has either become dormant through long continued inactivity or has been destroyed by an accumulation of waste products in the seed. It seems that a failure of specific enzymes to act may be one of the chief factors which limits the period of viability of seeds.

This view is in a measure confirmed by the behavior of some varieties of seeds, which have lost much of their germinating power through age, when soaked in solutions of enzymes capable of rendering the reserve food material of the seed soluble and available to the embryo. F. A. Waugh<sup>5</sup> obtained great improvement in the germination of old seeds when they were treated with solutions of various enzymes. Table V shows some of his results.

TABLE V. INFLUENCE OF ENZYMES UPON GERMINATION

Description of seeds	Solution employed	Per cent germination
Tomato, 12 years old.....	Water.....	12
Tomato, 12 years old.....	Diastase.....	85
Tomato, 12 years old.....	Water.....	34
Tomato, 12 years old.....	Diastase.....	70
Tomato, 12 years old.....	Water.....	14
Tomato, 12 years old.....	Diastase.....	24
Tomato, 5 years old.....	Water.....	36
Tomato, 5 years old.....	Diastase.....	46
Cucumber, 5 years old.....	Water.....	44
Cucumber, 5 years old.....	Diastase.....	54
Radish, 6 years old.....	Water.....	46
Radish, 6 years old.....	Diastase.....	66

Many other experiments with different seeds and a variety of enzymes most of which increased the germination, are described in Waugh's paper. In general, diastase improved germination more than other enzymes employed.

No doubt each variety of seed is supplied with particular enzymes, or combinations of enzymes, which serve its purpose better than is possible with any artificial preparation. It would

<sup>5</sup> Vt. Agr. Exp. Sta. Rpt., 1896. p. 106.



seem that diastase should be especially adapted to starchy seeds like corn, wheat, barley etc. while proteolytic enzymes such as pepsin and trypsin would be more beneficial to leguminous seeds and that lipase would serve the purpose best for seeds rich in fat.

A similar experiment to the above was conducted at this Station in November 1909, with corn, less than 50 per cent of which germinated when the seeds were soaked in water only. Seeds from the same lot and treated in the same manner as the checks, except that the water in which they were soaked contained commercial diastase, all germinated. The maximum growth was about the same in each lot, but the growth of the seeds soaked in diastase was very uniform while that of the water lot varied greatly. The increased vitality of the diastase lot was very noticeable.

Seeds from the same lot that were soaked fifteen hours in a 3 per cent solution of glucose instead of water, all germinated, thus confirming the view that lack of suitable food was the chief reason why the untreated seed germinated poorly. In this case, there was probably a lack of a starch-inverting enzyme in the seed since equally good results were obtained when either diastase or glucose was supplied; either would stimulate respiration, which once established, results in the production of the enzymes essential to the conversion of the stored nutrients into an available form.

It has been suggested that even immature seeds may contain all of the specific enzymes required for the changing of nutrients into forms that can be utilized by the embryo and that their action is prevented by certain anti-ferments which are present at this stage of growth but which are destroyed with the drying of the seed at maturity. It seems to me far more probable that these enzymes are wholly absent from a seed so long as it receives its nutriment from the parent plant and are only produced when direct and independent respiration of the embryo is established. It is difficult to explain on any other basis why the amount of these enzymes increases at such a rapid rate with the increased respiration that occurs during germination.

Whatever their source, the function of such enzymes is unquestionably to convert the reserve nutrients of the seed into available forms and thus maintain the life of the embryo until it reaches a state of development in which photosynthesis can

take place. It is even possible to continue growth for considerable periods, after the reserve material of the seed is exhausted, without photosynthesis, if proper nutrients are supplied to the embryo from external sources. This is shown by the experiments of Maze<sup>a</sup> who succeeded in growing vetches in darkness to a considerable size in sterilized nutrient solutions containing glucose. Some of his results are given in Table VI.

TABLE VI. ASSIMILATION OF CARBOHYDRATES BY VETCHES IN DARKNESS

Lot	Days of experiment	Per cent glucose supplied	Dry weight of seed, Mgs.	Dry weight of plant, Mgs.
1.....	50	1	202.8	269.0
2.....	39	2	202.8	276.7
3.....	92	4	202.8	838.2
4.....	92	6	202.8	710.0
5.....	53	0	202.8	161.6
6(a).....	53	0	202.8	133.4

(a) No nitrogen supplied.

The plants grown in the glucose solutions were much more vigorous than those in the check. Their tap roots were strong and well branched, and the stems attained a much greater length, amounting to as much as 1.3 meters, with branches extending 1.65 meters.

If the embryos be carefully removed from soaked grains of corn and placed in contact with starch paste, they will sprout and grow as freely as do those left in the unutilized seed.

These results indicate that the presence of specific enzymes is not essential to the growth of an embryo, if food material in suitable form is supplied from external sources.

*Distribution of Absorbed Water in Seed* When air dried seeds are immersed in water, they will in a few hours absorb more than their weight of water, and will increase considerably in volume. If the water in which the seeds are soaked has been freed from air by boiling, the seeds will become saturated without any signs of germination. The rate at which air dried seeds of corn absorb water, when immersed in it, and the distribution of this absorbed water between the embryo and the starchy portions is shown in Table VII. It seems likely that similar relations hold for all varieties of seeds.

<sup>a</sup> Compt. Rend., 1899, pp. 185-187. Abstracted in Exp. Sta. Record, Vol. XI, 1899-1900 p. 317.

It will be seen that the water taken up by the seed is not uniformly distributed between the embryo and the starchy portion and also that the embryo absorbs water more rapidly than other parts during the first 24 hours, after which the rate is

TABLE VII. EFFECT OF SOAKING CORN ON ITS WATER CONTENT  
Amount of water in the embryo and starchy portion of air dried corn compared with that in corn soaked for different periods in boiled water.

Air dried	Yellow Dent		White Dent	
	Percent water in embryo	Percent water in starchy portion.	Percent water in embryo	Percent water in starchy portion
.....	5.79	7.07	5.83	8.99
.....	5.59	7.08	5.65	7.11
.....	6.39	8.57	.....	.....
Hours soaked in water				
2.5.....	.....	.....	23.50	17.82
4.....	31.20	19.67	.....	.....
20.....	.....	.....	51.02	31.57
24.....	47.19	30.36	.....	.....
28.....	.....	.....	52.86	33.15
48.....	54.41	35.48	58.17	35.27
48.....	53.87	35.43	.....	.....
72.....	56.11	38.54	58.78	37.59
100.....	.....	.....	59.55	39.46

practically the same in all portions. The more rapid absorption by the embryo at first appears to be chiefly due to its peculiar structure and its location near the surface rather than to a more hygroscopic nature of its substance. The relatively low specific gravity of the embryo compared with that of whole kernels, shown in Table VIII made upon three separate samples of corn, suggests a more porous structure of the embryo.

TABLE VIII. SPECIFIC GRAVITY OF GRAINS OF CORN

Sample	Specific gravity of whole kernels	Specific gravity without embryos
1.....	1.2931	1.3449
2.....	1.2940	1.3135
3.....	1.2996	1.3639

The difference between the specific gravity of the embryo and that of the starchy portion of the kernel is really much greater than these figures indicate, since the embryo comprises only about one tenth of the whole kernel. No doubt the low specific gravity of the embryo is partly caused by the large proportion

of fat which it contains but it is probably due mostly to a more porous structure. If the substance of the embryo were more hygroscopic than other parts of the kernel, it should contain relatively more water than the starchy portion in air dried seeds, a condition which has not been observed in any determinations made upon corn. (See Table VII). Moreover the embryos, after being removed from the seed and dried, have in no case taken up water, when exposed to air, as rapidly as the starchy portions of the seed, but when immersed in water both parts behave as in the fresh seed.

The low percentage of water in the embryo of an air dried kernel of corn is doubtless due to the substance composing the embryo having, on the whole, a weaker affinity for water than does starch; the large proportion of fat, which has practically no affinity for water, in the tissues of the embryo is an important factor in determining this condition. The more rapid absorption of water when a seed is immersed in water is probably due to unoccupied spaces in the embryo and in the tissues immediately surrounding it, as these spaces are quickly filled with water. It is analogous to the absorption of water by a sponge, the substance of which may not in itself have a strong attraction for water. There is however abundant evidence to show that starch, which constitutes a large proportion of a kernel of corn, as well as of many other seeds, forms feeble molecular combinations with water analogous to those in crystals containing water of crystallization, and no doubt the same is true of the protein substances found in seeds. The water content of starch and corn that have been exposed to moist air is practically the same, indicating that the nature of the combination is the same in both cases.

#### CHANGES OCCURRING DURING GERMINATION

If the water content of a mature and air dried seed is raised to over 30 per cent and the seed is afterwards exposed to air or to free oxygen, at a suitable temperature, the vital activity of the embryo is enormously increased. This activity is manifested by a rapid absorption of oxygen, a corresponding evolution of carbon dioxide, an increase in the water content of the embryo, and a loss in the total dry matter of the seed. In a short time, which varies with the variety of seed, new cells are

formed and a sprout is pushed out from the embryo which finally develops into the root of the future plant. Shortly after the root is started, another shoot is formed which is destined to become the stem. This production of new tissue of a definite and characteristic form, from material within a seed, is designated by the term "germination."

#### CONDITIONS AFFECTING GERMINATION

A plentiful supply of water, a favorable temperature, and the presence of free oxygen are essential for the germination of all seeds.

*Water* The stored nutrients of a seed are mostly insoluble and consequently unavailable for the embryo until they are rendered soluble by combination with the elements of water. This change is effected by specific ferments, the production of which is dependent upon the direct respiration of the active cells of the embryo; it takes place very slowly except in the presence of considerable water. With corn the change is extremely slow when the water content is less than 20 per cent but the rate increases rapidly when the water content is raised above this; no sprouts appear, however, until the water amounts to about 25 per cent of the total weight of the seeds; above this point hydrolysis of stored nutrients proceeds with sufficient rapidity to supply all needs of the active cells of the embryo. Not only is water needed for this purpose but there must be provided, in addition, a sufficient quantity of water to dissolve the products formed and to transfer them by osmosis to the cells where respiration occurs. On the other hand, any excess of water above that which a seed can absorb is a disadvantage, since it removes soluble nutrients which are needed for the proper nourishment of the growing cells and also interferes with respiration by excluding oxygen. In consequence of this, a large surplus of water greatly weakens germination and may wholly prevent it.

*Temperature* The range of temperature within which germination may take place, depends upon the variety and condition of seed. Some seeds germinate in contact with melting ice at a temperature approximating 0°C., as is the case with wheat, but the seeds of most cultivated plants fail to germinate below 4° C. (39°F.) The optimum temperature for these plants is

about 30° C. (86° F), and germination is unsatisfactory above 40° C. (104° F.). The time required for germination is greater as the temperature is reduced below, or raised above the optimum.

*Oxygen* Although intramolecular respiration may occur with seed containing more than 10 per cent of water, when free oxygen is excluded, germination never takes place except under conditions that admit of direct respiration. Free oxygen is, therefore, essential although its presence in the gaseous state is not always necessary. Some seeds, when immersed in water are able to utilize dissolved oxygen, but none except those from water plants can make an extended growth under these conditions.

About twenty varieties of farm and garden seeds have been tested and all found to decompose hydrogen peroxide rapidly. When immersed in solutions containing this reagent, they were in most cases able to use the liberated oxygen for direct respiration; and germinated under these conditions as quickly as if exposed to air.

#### GERMINATION TESTS IN HYDROGEN PEROXIDE

The moisture, temperature and atmospheric conditions favorable to germination are also ideal for the growth of molds and other fungi that feed upon the soluble nutrients of a seed, and when such organisms gain the ascendancy they are sure to interfere seriously with the normal progress of germination. The most common organisms of this kind do not directly attack the living cells, but by withdrawal of nutrients and production of poisonous excretions cause a feeble growth and, if present in large numbers, especially in the early stages of germination, before photosynthesis is established, are sure to result in the death of the embryo.

When grown in soil under natural conditions, most healthy seeds overcome these attacks, but when grown in the laboratory between wet cloths or filters or even in moist air, molds and mildews are almost certain to appear and vitiate results, if an experiment is extended beyond three or four days. Attempts have been made during this investigation to avoid the growth of such organisms by using sterilized apparatus but even when this was done the spores adhering to the seeds were almost always sufficient to infect them and cause trouble before an ex-

periment was ended. No better success was attained when the seeds were subjected to antiseptics, as germination was always weakened and often entirely prevented, if treatment of the seed was sufficiently prolonged to destroy spores.

Among other reagents employed for this purpose was hydrogen peroxide, in which kernels of corn were immersed for periods ranging from twenty-four to seventy-two hours. The strength of the hydrogen peroxide solution used varied from  $\frac{1}{2}$  to 3 per cent. Seeds removed from any of these solutions, and exposed to air, germinated as well, apparently, as untreated seed and, if exposed in sterile vessels, were usually free from mold. Seeds kept in the solution from forty-eight to seventy-two hours germinated in the solution, with no direct contact with air, the oxygen required for respiration being derived from the reagent. When corn is germinated in this way, the sprouts grow to about  $\frac{3}{4}$  inch in length, after which the tip begins to curl and no further growth occurs, but if the seeds be removed from the hydrogen peroxide, even at this stage, and exposed to light, photosynthesis begins and growth is renewed.

Good results were obtained, with corn, with all strengths of solutions tried, the most favorable being with a  $1\frac{1}{2}$  per cent solution prepared by diluting the commercial 3 per cent solution with an equal volume of water.

The tests were made by placing a few kernels of corn, or other seeds, in a test tube or small Erlenmeyer flask with four or five times their volume of the solution of hydrogen peroxide. The tube or flask was partially closed by inserting a small test tube, containing water, in the mouth; this tube should reach below the surface of the liquid in the tube containing the seed. This arrangement permits the escape of oxygen as it forms and keeps the seed beneath the surface of the liquid. Some device of this kind is necessary as bubbles of oxygen adhere to the seeds causing them to float.

The best results have been obtained when the volume of the hydrogen peroxide solution was less than ten times that of the seeds tested. This is probably due to the removal of a large proportion of the soluble nutrients of the seed by the excess of water. It must be borne in mind that a sufficient supply of soluble organic nutrients, of the right kind to maintain respiration, is as essential to germination as is a supply of oxygen.

A very satisfactory method of making germination tests is to place the seeds between filter papers that are afterwards moistened with a 1½ per cent solution of hydrogen peroxide. In this way a large excess of the reagent is avoided and growth of parasitic organisms prevented. It is well in this case to renew the solution after twenty-four hours, the surplus liquid being poured off or absorbed by dry filter paper. In general, small seeds such as tobacco, timothy, clover, etc., have not germinated as readily with hydrogen peroxide as when water only was employed. Good results have been obtained with corn, wheat, rye, barley, buckwheat, peas and beans either when immersed in the reagent or when placed between filter papers and moistened with it. Oats have not germinated well, by either method, unless the hulls were previously removed; when this was done oats germinated as well in hydrogen peroxide as between wet filters. It has also been noticed that corn of low vitality, as shown by a low percentage of germination, requires a longer time for germination in hydrogen peroxide than in water. This suggests that the method may serve the purpose of discriminating between doubtful and good seed.

It is noteworthy that immature corn which failed to germinate in wet filters germinated perfectly after immersion for two weeks in hydrogen peroxide.

#### DISTRIBUTION OF WATER IN GERMINATING CORN

Kernels of corn, that have been immersed twenty-four hours or longer in boiled water free from air, do not germinate, although they absorb sufficient water to cause germination when oxygen is supplied. So long as oxygen is withheld from such seeds, the absorbed water remains distributed between the embryo and the starchy portion of the kernel in the manner shown in Table VII. If, however, oxygen is supplied freely the percentage water content of the embryo increases rapidly as germination proceeds and when sprouts are formed these are far more succulent than the embryo. During this period the per cent of water in the starchy portion of the kernel is but slightly reduced.

During the past two years, a number of tests have been made to determine these changes in the distribution of water in kernels of corn during the early stages of germination. Two varie-



ties of corn, a white and a yellow dent, grown upon the station grounds in 1909 and 1910 were used in these tests. In each test kernels nearly uniform in size and appearance were selected from the same ear and soaked for twenty-four hours in about ten times their volume of water that had been previously boiled to expel air. From a portion of these soaked kernels, freed from adhering water by contact with dry filter paper, the embryos were carefully removed and water determinations made separately in both parts, by drying in a steam oven at approximately 97° C. Other kernels, from the same lot of soaked corn, were exposed to nearly saturated air without being in con-

TABLE IX. EFFECT OF GERMINATION ON WATER IN SEEDS

Distribution of water and dry matter between the embryos and starchy portions of grains of corn, before and during the early stages of germination.

Condition of corn when tested		Whole grain	Embryo	Starchy portion
I. Air dried. ....	Per cent water.....	6.90	5.62	7.10
	Per cent dry matter.	93.10	94.38	92.90
II. Soaked 24 hours in boiled water.....	Per cent water.....	35.97	54.24	32.10
	Per cent dry matter.	64.03	45.76	67.90
III. Soaked grain after 24 hours in germinator	Per cent water.....	37.53	62.13	31.40
	Per cent dry matter.	62.47	37.87	68.60
IV. Soaked grain after 48 hours in germinator	Per cent water.....	38.32	65.82	31.03
	Per cent dry matter.	61.68	34.18	68.97

tact with a wet surface. Under these conditions sprouts grew to between  $\frac{1}{4}$  and  $\frac{1}{2}$  inch in the first twenty-four hours, and to about one inch in forty-eight hours. At each of these periods water determinations were made in the sprouted embryos and in the starchy portions of the kernels in the same manner as in the soaked seed. Very satisfactory and uniform results were obtained at these three stages, but when the germinated kernels were kept in moist air for another period of twenty-four hours, there was usually a considerable difference in the length of sprouts upon different kernels and often molds also appeared, which vitiated results.

The average per cent of water found in the different parts of the kernels, as well as its distribution between the embryos and starchy portions, for the first forty-eight hours of germination, are given in Tables IX and IXA.

Water content of sprouts, roots and stems after separation from the embryo, has ranged from 84.80 per cent to 90.17 per cent, and has averaged 87.75 per cent.

It will be noted that the per cent of water is higher in the germinated grain than in the soaked grain, and also that the per cent of water has increased as the period of germination has been extended, although the seed has at no time, during this period, been in direct contact with water. The most striking change, however is in the water content of the embryo which has been greatly increased by germination, in fact the

TABLE IX A. GRAMS WATER IN PARTS OF SEEDS

Distribution of water and dry matter between the embryo and starchy portion of grains of corn in different conditions—Calculated for 100 grams of seed.

Condition of corn when tested		Embryo grams	Starchy portion grams
I. Air dried.....	Water.....	.77	6.13
	Dry matter....	12.94	80.16
	Total weight..	13.71	86.29
II. Soaked 24 hrs. in boiled water .....	Water.....	9.45	26.48
	Dry matter....	8.02	56.01
	Total weight..	17.51	82.49
III. Soaked grain after 24 hrs. in germinator .....	Water.....	12.34	25.19
	Dry matter....	7.51	54.96
	Total weight..	19.85	80.15
IV. Soaked grain after 48 hrs. in germinator.....	Water.....	13.79	24.53
	Dry matter....	7.15	54.53
	Total weight..	20.94	79.06

whole increase in water content has taken place in this portion of the seed, since the per cent of water in the starchy parts has steadily diminished as germination has advanced.

The increase in the water content of the embryo, and the decrease in the starchy portion, might be explained by a direct transfer of water from the starchy parts of seed to the embryo, were it not for the increase in the water content of the whole seed, in spite of a continual loss of water, through respiration, the most of which has been from the embryo, where respiration is most active, and also through fixation of water in the hydrolysis of starch and other stored nutrients. It is possible that some water has been absorbed from the nearly satu-

rated air, but corn containing as much water as did the soaked kernels in the above tests takes up more water very slowly, unless it is in contact with a wet surface, and even then the embryo does not absorb water much if any more rapidly than other parts of the seed. It may even be questioned whether sufficient water is absorbed from saturated air, by a germinating seed to replace that lost by transpiration.

Tests were made to determine the rates at which corn, containing different per cents of water, increases in water content when exposed to saturated air. Only one variety of corn, a white dent, was used in these trials. The results are shown in Table X.

TABLE X. WATER IN CORN IN SATURATED AIR

This shows the absorption of water by corn exposed to saturated air.

Days exposed	Water content	
	Lot I %	Lot II %
0.....	8.42	8.42
1.....	15.44	14.25
3.....	22.01	20.61
16.....	25.51	24.52
8.....	26.06	25.90(a)
0.....	26.70(a)	.....

(a) At this time sprouts were starting upon both lots.

It will be seen that corn absorbs water quite rapidly at first, then slowly until the water content is sufficient to cause germination, which starts at a little below 30 per cent.

It is doubtful, in view of this, if the percentage increase of water in the whole seed can be attributed to absorption from air, or if the great increase of water in the embryo is due to a direct transfer from the starchy portion. The most satisfactory explanation both for the increased per cent of water in the whole seed and for its uneven distribution as germination proceeds, is the production of metabolic water by oxidation of organic matter, incident to respiration of the embryo. This view is sustained by a considerable loss of organic matter, especially in the embryo.

It is well known that the respiration of a germinating seed is chiefly due to the vital activity of protoplasm, within the embryo. In consequence of this, the easily oxidized carbohydrates

contained in the embryo are the first to disappear, being converted into carbon dioxide and water before other parts of the seed are attacked. The carbon dioxide produced soon escapes into the air, while the water, for the most part, remains in the tissues of the embryo where it is formed.

This local substitution of water for organic matter reduces materially the concentration of the cell fluids thereby disturbing the osmotic equilibrium between the embryo and other parts of the seed. In consequence of this, there is a diffusion of organic matter into the live cells of the embryo, and to a considerable extent also of water in the opposite direction, but since organic matter within the embryo is continually destroyed by oxidation, or removed from solution by being converted into insoluble nutrients or permanent tissues of the plant, the concentration of the fluids of a growing cell remains constantly less than that of the fluids in other parts of a seed. Organic nutrients must therefore continually move towards the embryo so long as the protoplasm is active, and water, metabolic water, arising from the oxidation of these nutrients, must also accumulate at this point. It is chiefly this local production of water within the growing cells that induces turgidity and pressure upon which the growth of new cells depends, rather than upon a movement of absorbed water, by diffusion, towards the growing points.

If a germinated kernel of corn, upon which sprouts have developed to a length of from one to two inches, be exposed to diffused light in moderately dry air, that part of the sprout next to the seed soon withers and apparently dies while the sprout remains succulent and continues to grow at the tip for several days. It is improbable that there is a transfer of either water or organic nutrients, through the dead tissue, from the seed to the living part of the sprout. The more active cells towards the tip of the sprout derive their nutriment partly from the older cells near the base which then die of starvation, and partly from slow photosynthesis. The water already in the stem, together with the metabolic water derived from the oxidation of these nutrients, is sufficient to maintain the vital functions, in a portion of the cells, for a considerable time, in spite of a constant loss of water incurred through evaporation and respiration, and of water used in photosynthesis during the day. The dead section becomes longer and longer as the

time of exposure is extended until finally the whole sprout is dry and dead, but so long as the tip remains alive, its water content is maintained at about 80 per cent. The high per cent of water maintained in the living cells is unquestionably due, in large measure, to metabolic water produced by oxidation of organic matter that is transferred by osmosis at first from the seed and later from the lower portions of the sprout. The gradual increase in the percentage of dry matter in the starchy portion of a seed, coincident with an increase of water in the embryo (See Tables IX and IXA), as germination proceeds, might be explained by transfer of water from the surrounding tissues into the embryo, or by a movement of organic matter in the opposite direction, but a consideration of the physical principles involved, and of the chemical reactions known to occur during germination, render this highly improbable. There is every reason to believe that the prevailing movement of free water, in a germinating seed, up to the time that photosynthesis begins, is away from, rather than towards, the embryo; and that the excess of water in the embryo is due to local oxidation of organic matter brought into it by osmosis, and not to direct diffusion of water from surrounding tissues.

The percentage increase of organic matter in the starchy portion of a seed, at this time, may be explained by the hydrolytic reactions that always occur in this part of a germinating seed, whereby water unites with starch and other insoluble carbohydrates, as well as with insoluble proteins, to form soluble and diffusible products. These reactions necessarily precede diffusion and in the early stages of germination must increase the per cent of organic matter in these tissues, especially if direct absorption of water from contact with a wet surface is prevented, as was the case in these trials.

This condition must prevail so long as hydrolysis of the reserve nutrients of a seed proceeds at a more rapid rate than diffusion of organic products to the embryo occurs, since oxidation of organic matter does not take place in the starchy portion of a seed. In consequence of this, there is, in the early stages of germination, an increase in the absolute as well as in the percentage amount of organic matter in the starchy portion of a seed.

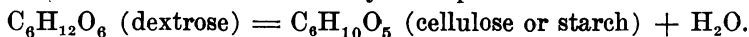
A further result of these reactions is a permanent difference between the concentration of soluble nutrients in the fluids of

the embryo and in the fluids of other parts of the seed, the concentration always being less in the embryo. This difference necessarily causes a movement of these nutrients by osmosis towards the embryo, where they are either oxidized, or deposited as new tissue containing a lower per cent of water. No matter which direction the reaction takes, the result is an accumulation of water in the growing cells.

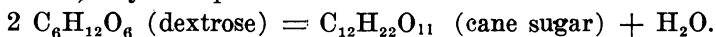
The metabolic water resulting from these reactions is considerable and, in spite of a transfer of water to other tissues, and of evaporation into the air, the sprouts growing upon the soaked seed contain between 80 and 90 per cent of water. This large excess of water in the growing sprouts, is not due to capillarity, since with no external water supply the capillary forces must be in equilibrium and the tendency for movement be equal in both directions; nor can it be caused by the more hygroscopic nature of the substances composing the cells of the sprouts, or their contents, for if respiration be suspended by exclusion of oxygen, by the action of poisons, or of anaesthetics, by heating or by freezing, the growing sprouts by reason of the thinner cell walls dry much more quickly upon exposure than do the original tissues of the seed. It appears therefore, that the accumulation of water in the active cells of a sprouted seed is independent of either the physical structure or the chemical nature of the tissues, but is most intimately associated with direct respiration, a vital process manifested by the absorption of oxygen and the evolution of carbon dioxide.

Since respiration is a function peculiar to living protoplasm, the carbon dioxide evolved must be derived from the oxidation of organic nutrients that are within the respiring cells, the only place where active protoplasm is found. In addition to the carbon dioxide evolved, there is produced, at the same time, within these cells a considerable quantity of water, the amount depending upon the nature of the substance oxidized and the completeness of the reaction. In a germinating kernel of corn, the oxidized substance consists chiefly of dextrose which has been derived from the stored starch of the seed by the action of diastase. The complete oxidation of dextrose results in the production of water amounting to 60 per cent of the weight and to nearly the same volume as the crystallized dextrose. But the amount of water resulting from the complete oxidation of sufficient dex-

trose to account for all of the carbon dioxide evolved, by no means represents the total water produced within these respiring cells, since this reaction, in a growing cell, is always associated with other reactions that not only remove dextrose from solution but at the same time liberate water. Thus cellulose is always produced within these cells and frequently starch is also formed, both of which are derived from dextrose. If only the initial substance and the final products are considered, the reaction in both of these cases may be expressed as follows:



Besides starch and cellulose, cane sugar is always found in varying amounts in the growing sprouts of corn, and this also must have originated from dextrose. In this case, only half as much water is liberated as when cellulose or starch are the products. The final result, when cane sugar is derived from dextrose, may be represented as follows:



Reactions analogous to these in which no carbon dioxide is liberated are always taking place not only in the sprouts of a germinating seed but in all tissues of plants, in all stages of development.

There is no way of determining how many times the same carbon nucleus may function as a carrier of water in these ways. It is evident, however, that metabolic water liberated within the respiring cells by the oxidation and dehydration of organic nutrients brought to them by osmosis, is a most important and probably the chief factor concerned in the movement of water from the leaves where organic nutrients are primarily formed, to the growing centers. It is amply sufficient, so long as proper nutrients are supplied, to not only maintain the high water content of these tissues, but also to induce sap pressure and turgidity in spite of considerable losses by evaporation and by diffusion of water to other portions of a plant.

The conditions prevailing in sprouting seed, which receives no water from an external source, are all opposed to a direct transfer of liquid water from the dead portion to the growing sprouts.

In a capillary system the liquid comprising the sap must be in equilibrium; the concentration of the nutrient solution is far greater in the older tissues where the reserve nutrients are

made available than in the growing sprouts where oxidation and deposition continually occur; and the sap pressure in the active cells is always high compared to that in the dead tissues of the seed. Under these conditions water must naturally flow away from rather than towards the respiring cells, if the movement depends solely upon physical forces and the water is transferred in the liquid state only.

On the other hand, every condition favors a movement of soluble organic matter in this direction by osmosis and all of the observed chemical changes in the seed and sprouts are in accord with it. In the respiring cells these organic nutrients are oxidized or dehydrated, sufficient water being liberated to supply all needs, so long as nutrients are available and evaporation is not excessive.

#### NUTRIENTS AND GERMINATION

Among the conditions essential to germination is a sufficient supply of suitable organic nutrients, in a soluble form, within a seed, to maintain respiration of the active cells of the embryo. As a rule, air dry, viable seeds contain a considerable excess of such nutrients which are mostly insoluble and unavailable, until water is provided for their solution and distribution. When a seed is placed in contact with about half its weight of water, or is surrounded by moist soil, it absorbs enough water for these purposes and respiration is greatly stimulated by the increased food supplied to the embryo. If however a seed be immersed in a larger quantity than it can absorb, some of its soluble nutrients are lost by diffusion into the excess of water, and when the amount of water is very large, compared to the weight of the seed, the soluble nutrients remaining in the seed may be too small to support normal respiration and the seed will either send out feeble sprouts, or may not germinate at all. If the water surrounding a seed be constantly renewed, as may happen during a prolonged rain, soon after planting, all of the soluble nutrients will be washed out and the seed will fail to germinate; in this case, germination fails because of a lack of sufficient soluble nutrients in the embryo to support respiration. The following experiments confirm these statements:

A sample of corn from a lot that germinated perfectly, in contact with moist filters, was placed in a flask with about



twenty times its weight of boiled water; the flask was frequently agitated to insure distribution of extractive matter and after a few hours the water was poured off and replaced by a fresh portion. This renewal of water was repeated six times in forty-eight hours. The first extract reduced Fehlings solution to a considerable degree and each subsequent extract less, the last having very slight effect, indicating that nearly all soluble carbohydrates had been removed from the corn. At the end of forty-eight hours, some of these soaked kernels were placed between moist filters for germination; after twenty-four hours, none of these kernels were germinated, but after forty-eight hours 80 per cent were germinated with weak abnormal sprouts, and no further germination occurred. Seeds from the same lot that were soaked for seventy-two hours in just sufficient water to cover them, all germinated with strong healthy sprouts after only twenty-four hours exposure to air in moist filters.

Another portion of the same corn was soaked twenty-four hours longer, seventy-two hours in all, and then tested for germination in filters moistened with pure water, in filters moistened with a 5 per cent solution of dextrose, and in a similar solution of dextrose to which a little active diastase was added. The results are shown in Table XI.

TABLE XI. GERMINATION OF CORN SOAKED IN BOILED WATER  
The sprouts upon the seeds that were germinated with water only were far weaker than those that received dextrose in addition.

Hours soaked	Hours of germination test	Per cent germination		
		In water	In dextrose	In dextrose and diastase
0.....	48	100	.....	.....
48.....	24	0	.....	.....
48.....	48	80	.....	.....
72.....	30	40	60	70
72.....	48	60	100	100
72.....	72	60	.....	.....

#### METABOLIC WATER IN MATURE PLANTS

Reactions by which the elements of water are tied up in organic combination to be liberated again in the liquid state, at the points where and when needed, are not confined to germinating seeds but occur in all stages of growth, in every variety of plants and provide an efficient means for the storage of water

in a non-volatile but immediately available form; they are the chief factors in the movement of water and of organic nutrients from one part of a plant to another; they induce turgidity and sap pressure in the growing shoots, where respiration is active, even after all connections with an external water supply are broken, and thus they enable a plant to withstand long periods of drought, or other adverse conditions, without permanent injury. These are all most important functions upon which the life and growth of plants depend and it is questionable if vital processes would be possible in multicellular plants without some such provision for the conservation and transfer of water from cell to cell.

The reactions involved in these transformations are all extremely complex and the intermediate stages obscure. In a plant they occur with equal facility in both directions hydrolysis and dehydration taking place even in the same cell at different times under slight changes in conditions which at present are not well defined. A few hydrolytic reactions of a similar nature to those that occur in plants, such as the conversion of starch into dextrose, may be brought about in the laboratory by chemical means; but up to the present time very few of the well recognized carbohydrates, or fats, and none of the proteins have been produced artificially, by dehydration or other treatment of a more highly hydrated member of the group to which it belongs.

#### COMPOSITION OF PLANT TISSUES

The dry organic tissues of plants consist chiefly of carbohydrates, fats and proteins, but as fats are primarily derived from carbohydrates, and in the metabolism of plants are probably converted into carbohydrates and since there appears to be no destructive metabolism of proteins, it is sufficient for the purpose of this paper to consider only the relations of the carbohydrate group.

*Carbohydrates* All carbohydrates consist of carbon combined with oxygen and hydrogen, the amounts of oxygen and hydrogen having the same relation to each other as in water. The first well defined and stable carbohydrate to appear in plants is starch, which is formed in the chlorophyl bearing cells of leaves by the action of light upon a solution of carbon dioxide

in water. If light is excluded, or in its presence if carbon dioxide is withheld, the starch previously formed in a healthy leaf soon disappears, being converted by specific enzymes, into dextrose and other soluble and diffusible carbohydrates of a higher degree of hydration than starch, which are distributed throughout the plant by osmosis and serve as nutrients for the active cells. It seems likely that this inversion of starch occurs continually, even in sunlight but that under these conditions, its effect is masked by the constant production of starch from carbon dioxide and water.

In the course of these transformations a great variety of carbohydrates is formed, the most sharply defined of which are cellulose,  $(C_6H_{10}O_5)_n$ , starch,  $(C_6H_{10}O_5)_n$ , cane sugar,  $(C_{12}H_{22}O_{11})$ , maltose  $(C_{12}H_{22}O_{11})$ , dextrose and levulose,  $(C_6H_{12}O_6)$ .

Aside from the above mentioned carbohydrates, there are a number of intermediate and closely allied bodies such as dextrine, the various gums, the pectous substances, the pentozans etc., the molecular structures of which are not well understood, but which have an important rôle in the carbohydrate metabolism of plants. No doubt in plants, some of these are always formed in every transformation of one of the principal carbohydrates to a higher or a lower degree of hydration, since they appear in every stage of plant development.

*Cellulose* This is the most stable of these carbohydrates and when once deposited, in a healthy plant, remains permanently in the place where it was formed, constituting an organic framework which supports the growing cells and determines the general form of the plant. It appears to be produced, in the plant, by a partial dehydration of dextrose, and other soluble carbohydrates derived from starch, a reaction that is always associated with respiration of the cells. It is not acted upon by any of the enzymes of healthy plants. It is possible, by the action of proper reagents, to hydrolyze cellulose and produce some of the more highly hydrated members of the carbohydrate group, but the reaction is never complete and the theoretical amount of dextrose is never obtained, nor is starch or cane sugar formed among the products of the reaction. It is insoluble in water or in the sap of plants.

*Starch* The same empirical formula is given to starch as to cellulose. Like cellulose it is a dyhydration product of solu-

ble carbohydrates. It is insoluble, either in pure water or in the sap of plants, and is the form in which reserve carbohydrate nutrients are most frequently deposited in the plant tissue. It is readily converted by diastase into the theoretical amount of dextrose, the reaction taking place with equal facility, within or without the plant. The same change may be brought about in the laboratory by the action of suitable reagents and is therefore not dependent upon vital processes. The reverse reaction has never been accomplished outside the plant. It is also possible that, in a plant, cane sugar may be formed directly from starch.

*Cane Sugar* This carbohydrate stands intermediate between starch and dextrose in the amount of hydrogen and oxygen, (the elements of water), that it contains. It is found in every part of most chlorophyl bearing plants, in every stage of their development, from the sprouting seed to the mature fruit. This indicates that it is a direct product of respiration, being formed, in some unexplained manner, in all active cells, from the dextrose or maltose that results from the hydrolysis of starch. It may serve as a reserve carbohydrate nutrient in an analogous manner to starch, and like starch cannot be directly utilized by cells as a source of energy for maintaining vital activity until it has been hydrolized and converted into invert sugar, maltose, or some other easily oxidized carbohydrate. This change may be brought about in any tissues of a plant but is usually effected in the leaves where conditions appear to be the most favorable, especially when exposed to light.

This function of leaves, by which cane sugar is hydrolized during photosynthesis is shown by the low per cent of cane sugar found in leaves, compared to that in other tissues, as well as by its high per cent in the sap of sugar-producing plants, such as sugar cane, Indian corn and sugar beets, as maturity approaches and the leaves become less active. Similar conclusions may be drawn from the observations of Keitt<sup>7</sup> upon the sugar content of sweet potatoes, who found more cane sugar in potatoes harvested after a wet and cloudy period than after a period of fair weather. The cane sugar that accumulates at maturity, and during resting periods when photosynthesis is

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<sup>7</sup> Bul. 156 S. C. Exp. Sta.

suspended, usually serves as a reserve nutrient and disappears when leaves again appear. Thus the cane sugar stored in the beet root, at the end of the first seasons growth, rapidly disappears when the leaves form in the following spring. The slow respiration that occurs during winter, in the cells of most deciduous trees, converts a large part of the stored starch into cane sugar which is inverted and made available as soon as leaves expand in spring.

Cane sugar diffuses, as it is formed, from the active cells into the vessels which transfer water from the roots to the leaves and for the most part, is carried by this current of water, together with carbon dioxide and other products of respiration, to the leaves, where all are converted by photosynthesis, into available nutrients and returned by osmosis to the active cells. This cycle may be repeated an indefinite number of times.

In this way, cane sugar and similar carbohydrates serve by means of alternating hydrolytic and dehydrating reactions, as most important agents in the transfer of water from the leaves to all of the growing cells of a plant. The amount of water transferred in this manner is unknown, since no means are available for determining the number of times which the same carbon atoms function in this rôle. I believe that the water abstracted from leaves, in this way, is nearly as potent a factor in causing leaves to wilt in sunlight as is direct transpiration of water from the leaves.

*Maltose* The same empirical formula ( $C_{12}H_{22}O_{11}$ ), represents maltose and also cane sugar; it is formed, with dextrose, by the action of diastase upon starch; it is soluble in water and in the sap of plants; it reduces Fehlings solution readily. Maltose is quite widely distributed in the vegetable kingdom and undoubtedly serves as a direct nutrient for the support of respiration; in this respect it differs radically from its isomer, cane sugar, since this is only available after inversion by ferments or acids to dextrose and levulose.

*Dextrose and Levulose* These carbohydrates are similar in their chemical relations and represent the highest state of hydration of any that occurs in plants. Both are soluble in water, are readily diffusible, and reduce Fehlings solution. It is in these forms that carbohydrate nutrients are most frequently transferred from place to place, in a plant, although cane sugar

serves this purpose to a limited extent. They also serve as important agents for the transfer of water to the growing cells. In a living plant, all other members of the carbohydrate group may be derived from them by dehydration and, conversely, all other carbohydrates except possibly cellulose may be converted into these by hydrolysis.

The importance of these properties of carbohydrates by which they may be changed from one degree of hydration to another, alternating between starch on the one hand and dextrose or levulose on the other, is illustrated by the transformations, already mentioned, that precede and accompany the germination of seeds. There is first, the absorption of water from an external source, in sufficient quantity to permit the action of enzymes upon the stored nutrients and for the transfer of soluble products by osmosis to the active cells of the embryo, where they stimulate respiration and are in part oxidized to carbon dioxide and water, and in part dehydrated to form lower members of the carbohydrate groups, containing less of the elements of water than before. In either case the organic nutrients that are brought to the respiring cells are partly replaced by water, so that the solution of nutrients within these cells is constantly maintained at a lower concentration than in the surrounding tissues. In this way conditions favorable to a continuous movement of nutrients towards the depleted centers, where growth occurs, are maintained, so long as the cells remain alive and a store of suitable nutrients is available.

#### BULBS AND TUBERS

All bulbs and tubers undergo transformations similar to the ones which seeds undergo; they constantly respire, absorbing oxygen, evolving  $\text{CO}_2$ , and producing water within the tissues. Respiration occurs chiefly at the growing centers, as in the central bud of an onion, or in the eyes of a potato. The enzymes, located at or near these centers act upon the stored food products, in the bulb or tuber, converting them into soluble and diffusible substances, which in turn are oxidized in the developing cells, thereby raising their water content and increasing their turgidity to a point where growth is induced.

If a bulb like an onion be immersed in water which has been boiled to expel air, it does not sprout, nor does it appear to

absorb water. If, however, the bulb be exposed to warm, moist air, it soon sprouts and grows to a considerable extent upon the stored nutrients which the bulb contains. In this case the sprouts contain a higher proportion of water than was originally present in the bulb. The excess of water in the sprouts is chiefly due to metabolic water formed from the organic nutrients by respiration. The fleshy tissues of a bulb serve not only to supply nutrients to the developing bud, but also to protect it from a too abundant supply of oxygen, until conditions are favorable for growth.

A similar provision is found in fruits, in which the pulpy material protects the seeds from free access of oxygen and reduces respiration of the seeds to a point where germination cannot occur until the easily oxidized constituents which surround them are destroyed. In most cases, this maintains the seed in a nearly dormant condition until the following spring, when germination occurs quickly and the plant has a whole season to develop and mature its woody tissues and buds before winter. Were it not for this protection, many seeds would germinate in late summer and the immature growth would be killed by freezing. The influence of the pulpy substance of fruit, in delaying germination of seeds is seen in melons and similar fruits the seeds of which do not germinate so long as the fruit is unbroken, although temperature and moisture conditions may be ideal for growth, but when seeds are removed from the fruit and placed in moist soil, or between wet filters, in contact with air, they germinate quickly. Even when the fruit is broken so that air has free access to the seeds, some seeds germinate in a short time, if molds are suppressed, showing that the one condition, essential to growth, that is absent, is a supply of oxygen. Germination of fresh seeds from pulpy fruits like melons is facilitated by thoroughly washing the seeds to remove the adhering slimy material; unless this is done molds quickly appear in abundance and prevent germination. The best results have been obtained when the washed seeds were placed between filter papers that were moistened with a 3 per cent solution of hydrogen peroxide, which supplies oxygen in abundance and keeps molds from gaining the ascendancy, until after the sprouts start. Treated in this way the fresh melon seeds germinate nearly as quickly and as well as dried seeds.

## VIABILITY OF IMMATURE SEEDS

Seeds seldom germinate while they are attached to the succulent living tissues of the parent plant, although temperature and moisture conditions are usually favorable at this time. The failure to grow may be due to exclusion of free oxygen by the seed envelope, and it may be that organic nutrients, in a form suitable for the growing sprout, are absent at this time. Whatever the direct cause may be, the adverse conditions disappear after the immature seed is exposed to air for a period that depends upon its variety and maturity.

Radish seed, taken from green seed pods, all failed to germinate, when transferred directly from the pod to wet filters, but seed from the same lot, after being kept ten days in warm dry air, all germinated within forty-eight hours, under the same conditions.

Corn that was apparently mature, but picked while the husks were still green, behaved in a similar manner to the radish seed. Not a single kernel sprouted when tested immediately after picking. All grew after a preliminary ten days' exposure to warm, dry air. The same result was obtained with sweet corn, picked in an edible condition while the kernels were still soft and milky.

In the case of corn, this change in viability is not due to preliminary drying, since soft kernels from the same ears, when immersed in hydrogen peroxide, all germinated within two weeks, without having been dried at any time. This indicates that direct respiration is the chief factor in bringing about those changes in the seed, that are essential to germination. Further confirmation of this view is supplied by failure of such seeds to germinate after being kept, for a similar period, in carbon dioxide or in boiled water, where no free oxygen was available.

## DEVELOPMENT OF HYDROLYTIC FERMENTS IN SEEDS

Kernels of corn, from the same ears described above, were tested for diastatic ferments, when first picked, and again when germination tests were afterwards made. For this purpose, the crushed kernels were digested for a few hours in water to which a little toluol or chloroform was added to inhibit fermentation; to the clear filtrate from this mixture, sufficient starch, in solution, was added to give a faint but distinct blue color with iodine;



the solution was kept warm and tested with iodine, at frequent intervals. A control solution in water only, containing the same amount of starch, was exposed and tested in the same manner; if no difference in the intensity of the reaction, with these solutions, was observed after two hours, it was assumed that diastatic ferments were absent from the seed. In all tests made with corn, after it had been picked sufficiently long to germinate well, the starch reaction disappeared in a short time, indicating the presence of a starch inverting enzyme. In only one test of soft corn, that of an ear with the husks dry when picked, was any indication of a diastatic ferment found.

A summary of a number of these tests is given in Table XII. The water content of samples of corn tested is also given to show the relative maturity of the seed.

TABLE XII. GERMINATION OF MATURE AND IMMATURE SEEDS

Influence of maturity and exposure to air upon germination and upon the presence of a diastatic ferment in the seed.

Variety of seeds	State of maturity	Per cent water	Percent germination		Diastatic ferment
			In wet filters	In hydrogen peroxide	
Yellow dent corn....	Ripe, one year old..	8.50	100 in 48 hours.	100 in 48 hours.	Present in abundance.
Yellow dent corn....	Ripe but soft.....	40.62	0	100 after 14 days	Small amount
Yellow dent corn....	Ripe but soft.....	54.22	0	100 after 14 days.	Doubtful
Stowell's Evergreen sweet corn.....	In edible condition.	74.71	0	.....	None
Radish .....	From green pods....	.....	0	.....	.....
Radish .....	From same green pods as above after 10 days.	.....	100 in 48 hours.	.....	.....

After being exposed to warm dry air for ten days, seed from each of the ears tested as shown in Table XII germinated within forty-eight hours in wet filters, and also when immersed in a solution of hydrogen peroxide.

These tests indicate that the presence of certain specific enzymes is essential to the germination of seeds, and also that the production of such enzymes occurs only under conditions which admit of direct respiration. The great increase in respiration, as well as in the production of such enzymes at the time of germination gives support to this view.

The course of metabolism in an immature seed differs widely from that in a germinating mature seed. In the former case, soluble nutrients are being converted into insoluble reserve materials, while in the latter case the reactions are reversed, since the sprouting embryo must receive its nutrients in a highly hydrated and soluble form. It is not strange, therefore, that hydrolytic enzymes are wholly absent from immature seeds, so long as a surplus of suitable organic nutrients is supplied through the circulatory system of the parent plant, and that they are formed only after direct and independent respiration is established.

Unbroken seeds never germinate in the digestive tract of an animal, because free oxygen to support direct respiration is not available. In this case, temperature and moisture conditions are ideal for growth and when voided, in the excrement, and exposed to air, such seeds in general germinate quickly.

The absorption of water by seeds, the conversion of starch into dextrose by diastase, the distribution of dextrose by osmosis and diffusion throughout the water of the seed, and all other phenomena that precede germination take place with equal facility in dead and living seeds. In fact nearly all parts of a viable kernel of corn, or other seed, are dead and may be removed from the embryo without affecting the development of new tissue, provided proper nutrients are supplied from other sources.

The very slow action of diastase in converting starch into dextrose, under conditions prevailing in an air dried seed, and its greatly increased activity when an abundance of water is supplied are shown by the following experiment.

Five grams of coarsely pulverized corn meal were boiled in 100 c. c. of water to destroy the activity of the diastase contained in the meal. To another similar portion of the same meal was added 100 c. c. of cold water. A few drops of toluol were added to each to prevent fermentation, and both portions were left at room temperature for twenty-four hours. The filtrate from the cold extract, when boiled with Fehlings solution, gave a copious precipitate of cuprous oxide, while scarcely a trace appeared with that from the boiled meal. The boiled extract contained all of the dextrose that had accumulated during several months in the air dried seed, while the cold extract contained in addition, the amount of dextrose formed in only twen-

ty-four hours, by the same quantity of diastase, in the presence of sufficient water to insure its maximum activity.

#### MOLECULAR COMBINATION OF WATER WITH THE CONSTITUENTS OF SEEDS

The water absorbed by seeds before germination may be held by purely physical forces, such as are manifested in capillarity and adhesion, or it may be in molecular combination with some of the organic constituents of the seed as appears to be the case in the phenomena of imbibition. The removal of such water by exposure to a temperature not exceeding 100°C. suggests that physical forces only are involved, but the persistence with which seeds retain water, when exposed to dry air at ordinary temperatures, indicates that at least a part of the water is held by feeble molecular combinations analogous to that in crystals containing water of crystallization.

#### THE NATURE OF IMBIBITION

The term "imbibition" is used by botanists and plant physiologists to designate the phenomena associated with the absorption of water by the solid material of plants. The most widely accepted hypothesis concerning these phenomena is that first advanced by Naegeli<sup>8</sup> that all organized tissues of plants, that are capable of imbibition, consist of minute molecular aggregations designated as micellae, between which water enters, forcing them apart thus increasing the volume of the tissues, when they are immersed in water. The entrance of water between the micellae is supposed to be effected by some other force than capillarity, since it does not take place when the tissues are immersed in such liquids as absolute alcohol or anhydrous glycerin.

This argument, which is advanced to show the inadequacy of capillarity to explain the phenomena of imbibition, appears to me to be equally conclusive against the existence of micellae. In order that a liquid may be absorbed by capillarity it is essential that the absorbing solid and the absorbed liquid have some affinity for each other. Most liquids will rise in a capil-

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<sup>8</sup> A clear presentation of Naegeli's views and the evidence in support of them is given by Sachs, (*Lectures on the Physiology of Plants*, translated by H. Marshall Ward, Lecture XIII), and Pfeffer, (*Physiology of Plants*, translated by Alfred J. Ewart, Chap. III).

lary tube of glass, to a point above the level outside, but mercury, which has no molecular attraction for glass, only enters a capillary tube when subjected to pressure. The same principle is involved in all phenomena of this nature, and all liquids which adhere to the clean surface of a solid will enter a capillary opening in that solid, or will be forced between the limiting surfaces of such solids when they are brought as nearly in contact as possible; the force that causes the liquid to enter these spaces is an inverse function of the distance between them. The fact that plant tissue does not swell when immersed in absolute alcohol, or anhydrous glycerin, both of which adhere to the clean dry surface of bodies composed of such tissues, is conclusive evidence of the inadequacy of Naegeli's hypothesis. On the other hand, all of the phenomena of imbibition point directly to a molecular combination between the substance composing an organized body and water. It is true that, in most cases, the combination is feeble, since it is broken up by a relatively low temperature without changing the molecular structure of either the solid tissue or the water. It is, however, entirely analogous to the behavior of many substances, both organic and inorganic, which crystallize with water of crystallization. Such substances exhibit the same phenomena in combining, viz. a change of volume, an evolution of heat, which is less for each additional increment of water, and a tendency towards a uniform distribution of water throughout the whole mass, when saturated and unsaturated portions are brought in contact.

For these reasons, it seems far simpler to account for the phenomena of imbibition by a direct, molecular combination of the substances composing the tissues of organized bodies and water, than by assuming the existence of micellae, the structure and form of which cannot be demonstrated.

#### CHANGE OF TEMPERATURE WHEN SEEDS ABSORB WATER

Further light on this question has been sought by determinations of the thermal balance, when seeds of corn are moistened with water under conditions which prevent vital activity. If there is no molecular combination, under these conditions, there should be no appreciable disturbance in the thermal balance when a seed absorbs water; if, on the other hand, the absorbed water is chemically combined with some of the organic constitu-

ents of the seed, it should be indicated by a change in temperature.

In order to differentiate the reactions involved in the preliminary absorption of water from those associated with vital processes, an antiseptic was added to the water with which the seeds were moistened. This not only suspended all vital activity of the respiring cells of the seed, but also prevented the growth of molds and other organisms during the test. On the other hand, the absorption of water and the hydrolytic action of enzymes upon the stored nutrients of the seed were not interfered with.

Two varieties of corn, a white and a yellow dent, both of the crop of 1910, were selected for the test.

In each test 150 grams of corn were placed in a silvered Dewar flask of 500 c. c. capacity with 200 c. c. of water, which is sufficient to cover the seed, and 5 c. c. of toluol to prevent fermentation and suspend respiration. A sensitive thermometer, graduated to  $0.1^{\circ}\text{C}$ . was inserted through a perforated rubber stopper and the temperature observed at frequent intervals. During the test the flask was kept in a room the temperature of which was approximately the same as the contents of the flask. Two experiments were made with each variety of corn. In each case the temperature rose quite steadily at first, reaching a maximum in eight to ten hours, after which it remained constant for the next 30 hours and then gradually fell off. The results were concordant, showing a maximum increase in temperature of  $1.7^{\circ}\text{C}$ . for the yellow corn, and of  $2.6^{\circ}\text{C}$ . for the white corn. Assuming that the specific heat of corn is 0.3, and that no heat was lost by radiation, the heat change, indicated by the maximum temperature is equivalent to 3.25 calories per gram for the yellow, and to 4.25 calories per gram for the white variety. To each of these values should be added the heat absorbed by the apparatus and the heat dissipated by radiation, during the test, the amounts of which are unknown.

At the end of the test the water content of the yellow corn was 46.94 per cent, and of the white corn 53.48 per cent. This difference indicates a higher degree of hydration of the white variety and explains, in a measure, the difference in the amount of heat set free.

These results give no clue to the particular constituent of the seed which united with water to cause the rise in temperature,

but the large preponderance of starch in the corn grain pointed to this as the most probable source.

Confirmation of this view was sought by comparing the amounts of water absorbed by starch and by the corn grain, under similar conditions, and also by measuring the heat evolved when starch was mixed with water. Previous experiments had shown that corn, when exposed to saturated air, absorbs water rapidly at first, then more slowly until, at the end of a week, it contains about 25 per cent of water. Changes incident to germination begin with about this proportion of water and cause the water content of a live seed to increase more rapidly after this time. In order to avoid the error incident to vital activity, even when the time was extended to complete saturation, the absorption of water by kernels of corn that had been dried at  $97^{\circ}\text{C}.$ , and were dead, was determined for comparison with starch.

About 5 grams of the dried corn was exposed to air that was saturated with moisture, under a bell glass. Complete saturation of the air was secured by suspending within the bell glass, which stood in a dish of water, strips of filter paper with the

TABLE XIII. GAIN OF WEIGHT BY CORN AND STARCH IN MOIST AIR

Percentage gain in weight and water content of dry corn and starch, when exposed for different periods to air saturated with moisture.

Days exposure	Starch		Corn	
	Per cent gain in weight	Per cent water content	Per cent gain in weight	Per cent water content
0.....	0	0	0	0
1.....	12.60	11.19	8.66	7.97
2.....	17.30	14.75	14.76	12.86
3.....	20.22	16.82	18.58	15.67
4.....	22.32	18.24	21.28	17.55
5.....	23.86	19.26	23.52	19.04
6.....	25.22	20.14	26.00	20.63
7.....	26.50	20.95	27.83	21.69
8.....			29.77	22.94
9.....	28.80	22.36	31.20	23.78
13.....	30.74	23.51	32.41	24.48
18.....	32.88	24.74	34.01	25.38

ends immersed in the water at the bottom. The corn was weighed at various intervals and the percentage water content calculated from the increase in weight. A similar method was used with starch that had been dried at  $110^{\circ}\text{C}.$ , 50 grams of the dry starch being exposed in a wide crystallizing dish. The room temperature during the experiments was approximately  $20^{\circ}\text{C}.$  The results of the tests are given in Table XIII.

On the fifth day and each day afterwards, when the starch was weighed, it was stirred with a glass rod to expose a fresh surface. The appearance of mold upon the starch brought the experiment to a close on the eighteenth day.

Results similar to these were obtained by Rodewald<sup>9</sup> by exposing between two and three grams of dry starch to a saturated air. The gain in his experiment was somewhat more rapid than shown in Table XIII, because of the relatively large surface of starch exposed to the air. The percentage gain in weight, and the water content of the starch, computed from his data, are given in Table XIV.

TABLE XIV. GAIN IN WEIGHT OF STARCH IN MOIST AIR

Percentage gain in weight of dry starch, and its water content after exposure to air saturated with water, for various periods.

Days of exposure	Percent gain in weight	Percent water content
0.....	0	0
1.....	14.55	12.70
3.....	24.62	19.75
5.....	28.25	22.03
11.....	32.42	24.48
24.....	32.54	24.55
75.....	32.60	24.59

The close agreement of the results obtained in these two independent tests, at the eighteen day period, in spite of the great difference in the quantities of starch used, indicates that the starch must have been nearly saturated with water at this time. This view is confirmed by the fact that practically no gain was observed during the next sixty days in Rodewald's experiments. At this time the starch contained nearly 25 per cent of water which, calculated for the empirical formula,  $C_6H_{10}O_5$ , of starch, is equivalent to three molecules of water. This close agreement to theory suggests that the absorbed water is in molecular combination.

Further evidence of such a union is supplied by the evolution of considerable heat when dry starch is mixed with water. The experiments of Naegeli<sup>10</sup> established this fact and later Rode-

<sup>9</sup> Ueber die Quellung der Staerke. Landw. Vers. Stat. 45, 1895, p. 201.

<sup>10</sup> Theorie der Gaehrung. 1879, p. 133.

wald<sup>11</sup> showed that the heat evolved when dry starch is mixed with water, at an average temperature of about 20°C. is equivalent to 23.4 calories per gram of starch.

Since the water absorbed by starch is readily expelled at a temperature of 100°C., the molecular combinations formed must be feeble; they appear to be closely analogous to those existing in crystals containing water of crystallization. The nature of this preliminary combination differs widely from that resulting when starch is converted into dextrose, in the presence of diastase, by adding on to its molecule the elements of water. In the latter case, the molecule is far more stable and cannot be broken up into starch and water by the application of heat. This difference is also shown by a greater evolution of heat, when starch is converted into dextrose, in spite of a much smaller quantity of water being required for the reaction. The theoretical amount of heat set free in this case, as well as in other reactions occurring when one carbohydrate is converted into another in a plant, may be calculated from the heats of combustion of the substances involved.

The heats of combustion of some of the more important carbohydrates found in, and associated with the nutrition of plants, are given herewith. The values selected are from the tables of Landolt and Bornstein and are the average of all results obtained at constant volume. Complete oxidation of one gram of cellulose produces 4192.7 gram calories (a gram calorie being the heat required to raise the temperature of one gram of water from 0°C to 1°C); of starch, 4205.2; of cane sugar, 3958.4; of dextrose and levulose, 3752.3 gram calories; of water and carbon dioxide, none.

Since the thermal balance of all reactions that occur in the conversion of one substance into another is always the same, no matter what course the reaction may take or how many intermediate steps may occur, it is possible to calculate, from the preceding data, the heat equivalent of energy absorbed or set free, when any one of these carbohydrates is changed into another. Thus by the complete hydrolysis of one gram of starch there is produced one and one-ninth grams of dextrose. When this amount of dextrose is oxidized to carbon dioxide and water,

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<sup>11</sup> Ueber die Quellung der Staerke. Landw. Vers. Stat. 45, 1895, p. 201.



there is liberated 4169.2 calories; the difference between this and the heat of combustion of the original gram of starch, (4205.2), is thirty-six calories which is the heat equivalent of the energy set free in the reaction. Conversely, when one and one-ninth grams of dextrose are converted into starch, energy equivalent to thirty-six calories must be supplied from external sources, if thermal equilibrium is maintained. The energy balance between any of these carbohydrates may be calculated in a similar manner.

The difference between thirty-six calories, the theoretical amount of heat evolved when one gram of dry starch is converted into dextrose, and 23.4 calories, the heat evolved, according to Rodewald, when dry starch is mixed with cold water, amounting to 12.6 calories should represent the heat set free, when starch paste containing an equivalent of one gram of dry starch is converted into dextrose by diastase. In order to test this, and thus independently to confirm the determination by Rodewald, a starch paste was prepared in the usual manner from a good quality of corn starch by pouring boiling water over it after it had been mixed with cold water to avoid a lumpy condition.

This paste was strained, a little toluol added to prevent fermentation, and placed in a constant temperature room for twenty-four hours, when its temperature was approximately the same as the room. It was thoroughly mixed by pouring from one vessel to another and 450 c. c., equivalent to about thirty grams of air-dried starch, was placed in each of two silvered Dewar flasks of 500 c. c. capacity. To the contents of one of these flasks, was added 50 c. c. of active malt extract, and to the other 50 c. c. of water, the whole being carefully mixed by shaking. A sensitive thermometer was placed in each flask and the temperature observed, at intervals. For forty-eight hours the temperature in both flasks remained between  $21.6^{\circ}$  C. and  $21.7^{\circ}$  C., the temperature of the room in the meantime, ranging from  $21.6^{\circ}$  C. to  $21.9^{\circ}$  C. During this period all of the starch to which malt extract was added had disappeared, being converted into dextrose. The experiment was repeated a second time with similar results, no appreciable change of temperature being observed in either test that could be attributed to the hydrolysis of starch.

Because of these negative results, it seemed advisable to repeat the tests of Rodewald, with starch from the same lot used in the preceding experiments, and under similar conditions. The starch used for this purpose lost 9.83 per cent in weight, when dried at 97°C. and 10.38 per cent at 110°C. This coincides approximately with the formula  $C_6H_{10}O_5 + H_2O$ , which is equivalent to a water content of 10 per cent. An estimation of starch in the substance dried at 110°C. gave a purity of 93.7 per cent.

In the first test fifty grams of the air-dried starch was placed in a silvered Dewar beaker and 100 c. c. of water having the same temperature as the starch, added to it. The starch and water were mixed to a smooth paste, by stirring with a sensitive thermometer, and the temperature observed. The temperature rose quickly and within five minutes reached a maximum 2° C. higher than the initial temperature. This was repeated a number of times, with practically the same results. Assuming the specific heat of starch to be 0.3, the heat liberated is equivalent to 230 calories; to this should be added the heat absorbed by the apparatus, which, under similar conditions, was found to be approximately fifteen calories for each degree of change in temperature; a total, in this case, of thirty calories. This makes a total of 260 calories set free when fifty grams of air dried starch containing 10 % of water was mixed with water, at a temperature of approximately 20° C. This is equivalent to 5.2 calories per gram.

In a similar trial with starch that had been exposed to air saturated with moisture, and which contained 25 per cent of water, heat equivalent to 0.93 calories per gram was liberated, indicating that the starch used was not entirely saturated with water.

The average amount of heat liberated, in three trials, in which fifty grams of starch dried at 110° C. were mixed with 100 c. c. of water, was equivalent to 21.8 calories per gram; calculated for pure dry starch it amounts to 23.3 calories per gram, a value agreeing closely with the results obtained by Rodewald.

It has been suggested that the rise in temperature, in these cases is due to simple absorption and not to a molecular union of the starch and water; this is unlikely since an equal amount of dry filter paper, immersed in water, under the same condi-

tions, gave no appreciable change in temperature, although the paper absorbed water more rapidly than did starch.

The foregoing results point directly to a feeble molecular combination of starch with water, analogous to that existing in crystals containing water of crystallization. This is well illustrated by the behavior of calcium sulphate, which crystallizes as gypsum with two molecules of water. Most of the water of crystallization is easily driven off at a temperature not much above  $100^{\circ}$  C. forming a white powder known as plaster of Paris, which when again mixed with water readily combines with it to form gypsum again, considerable heat being set free by the reaction. If gypsum be heated to between  $300^{\circ}$  and  $400^{\circ}$  C. instead of below  $200^{\circ}$  C., it loses all of its combined water and is changed into anhydrite, a form that no longer combines directly with water when mixed with it; it still imbibes water but no molecular combination occurs and no heat is evolved. In this condition it corresponds to cellulose which is not hydrolyzed by simple contact with water while plaster of Paris corresponds to dry starch which unites directly with water with an evolution of heat, and gypsum itself corresponds to hydrated starch as it exists in boiled starch paste.

The heat liberated, when dry starch is mixed with water, is about two thirds of the difference between the heats of combustion of starch and an equivalent amount of dextrose, and it was naturally expected that a further evolution of heat, sufficient to make up this difference, would be noted when starch paste was converted into dextrose by diastase. The negative results obtained in tests with starch paste indicate either, that heat was evolved in the preparation of the starch paste and escaped notice at the high temperature employed, or that the hydrolysis of starch by diastase is associated with other reactions which absorb sufficient heat to counterbalance the heat evolved in hydrolysis.

Among changes of this latter type is the absorption of heat when a solid substance is dissolved. In this case, the starch is almost wholly insoluble in water, while the resulting dextrose passes into solution as rapidly as it is formed. The heat absorbed during the solution of dextrose was determined by making the solution in a Dewar beaker in the same manner as that employed for obtaining the heat set free when dry starch is

mixed with water. The preliminary trials were made with commercial dextrose, containing 7.83 per cent of water, which is about 1 per cent less than the theoretical amount for one molecule of water of crystallization. Otherwise the sample was pure.

In two trials, made with this crystallized dextrose, the heat absorbed during solution was equivalent to 23.5 and 24.4 calories per gram, respectively, an average of 23.9 calories per gram.

Two other trials made with anhydrous dextrose resulted in an absorption of 13.25 and 13.27 calories per gram respectively, an average of 13.26 calories per gram. This is at the rate of 14.7 calories absorbed by the solution of the one and one-ninth grams of anhydrous dextrose that is derived from one gram of pure, dry starch.

These results indicate that the solution of dextrose takes place in two stages, the first being a combination of the anhydrous substance with water of crystallization; this is an exothermic reaction and heat is liberated. The second stage, in which crystallized dextrose is dissolved, is an endothermic reaction the heat absorbed being considerably more than the heat set free in the first stage. The heat absorbed when anhydrous dextrose is dissolved in water represents the difference between these quantities whereas the total effect of the endothermic reaction appears when crystallized dextrose is dissolved. The results obtained are entirely in accord with those that occur in the solution of all substances which unite with water when they crystallize. Thus anhydrous calcium chloride combines with six molecules of water in crystallizing with an evolution of considerable heat; on the other hand, the solution of crystallized calcium chloride absorbs sufficient heat to act as an efficient freezing mixture. The solution of substances like cane sugar, which crystallize without water, is always accompanied by an absorption of heat.

The thermal equilibrium that prevails when starch paste is hydrolyzed by diastase indicates that the heat liberated in this reaction corresponds in quantity to the heat absorbed when the resulting anhydrous dextrose is dissolved in water. If this is the case, the sum of the calories set free, when dry starch is mixed with water, and the calories absorbed when an equivalent amount of anhydrous dextrose is dissolved in water, should equal the difference between the heats of combustion of these quantities

of dry starch and anhydrous dextrose. It has been shown that this difference, for one gram of starch and the dextrose derived from it amounts to 36 gram calories. It was found that the heat liberated when dry starch is mixed with water, (23.3 calories), added to the heat absorbed when an equivalent amount of anhydrous dextrose is dissolved in water, (14.7 calories), equals thirty-eight calories per gram of starch, a difference of only two calories per gram between the theoretical value and that found by experiment. This value is based upon the amount of pure starch, found by analysis, in the sample used, the effect of impurities being ignored. It is possible, however, that the starch used in these experiments may have been as pure as that used in most calorimetric tests upon which the heats of combustion are based. If this is the case, the heat liberated, when water is added, should be calculated upon the total substance used instead of upon the pure starch which the sample contained, while the heat absorbed by the solution of dextrose should be calculated for an equivalent of the pure starch present. On this basis, the heat accounted for, in these experiments amounts to 35.5 calories per gram of the starch used, which is within half a calorie of the theoretical amount, derived from the heats of combustion.

These empirical values with starch, when considered in connection with the heat evolved by air-dry grain that is immersed in water, indicate clearly the course of the hydrolysis of starch in a germinating seed of corn. The first step is the direct union of starch with water, the combination being of the same nature as that in crystals containing water of crystallization. It is this reaction which causes the preliminary rise in temperature, when dry seeds are immersed in water, in the absence of oxygen. Then follows the hydrolysis of starch to dextrose, by diastase, in which the seed remains in thermal equilibrium; this change also takes place in the absence of oxygen, and is not associated with the evolution of carbon dioxide. The subsequent evolution of heat, that occurs with all varieties of seeds, when sprouts develop, results from the direct oxidation of dextrose, or of a similar carbohydrate, induced by the respiration of active cells situated in the embryo; it is always associated with an evolution of carbon dioxide.

The direct union of starch with water, in a feeble combi-

nation analogous to water of crystallization, serves a most important purpose in maintaining a sufficient amount of water in dormant seeds, exposed to dry air, to insure the continued activity of vital processes in the few respiring cells of the embryo. It is this molecular combination that makes seeds so retentive of water, and which enables germinating seeds to withstand drought without permanent injury.

#### RESERVE NUTRIENTS OF PLANTS

Unless each active cell receives a constant and sufficient supply of available nutrients from external sources to maintain respiration and other vital processes, the tissues of the cell are in part consumed and in a short time the cell dies from starvation. This event is guarded against by a store of reserve material in each cell, which is not used so long as available nutrients from an external source are supplied, but which may be utilized to support respiration for a limited time, when for any reason the usual supply fails.

The primary source of all organic nutrients is photosynthesis which occurs, for the most part, in the chlorophyl bearing cells of the leaves, from which the nutrients in a high state of hydration are distributed by osmosis to all of the active cells of a plant. In these latter cells the nutrients are in part oxidized, producing carbon dioxide and water, and in part dehydrated. Part of these metabolic products, as cellulose, starch, fats etc., are insoluble and remain in the cell either as permanent tissues, (cellulose), or as reserve nutrients, (starch and fats), to be drawn upon whenever the supply from the leaves fails; other parts are converted into soluble products, (cane sugar, organic acids etc., containing less of the elements of water than the original nutrients), which are not directly available as nutrients and are excreted from the active cells into the tracheids and other vessels that transfer water from the roots, and are carried by this current of water to the leaves, where they are again hydrolyzed and become available as nutrients.

These products, including even that part of the carbon dioxide which is dissolved in the sap, may, therefore, be considered to be reserve nutrient materials also, since the energy required for restoring them to an available form is supplied by photosynthesis, from an external source. In some plants, these

soluble products of respiration that have been excreted from the active cells are the most important of the reserve carbohydrate nutrients. This is the case with cane sugar in sugar cane, Indian corn, some fruits, and many roots and tubers at the end of the first year's growth.

Some of these reserve nutrients, especially the insoluble forms such as starch, the fats etc. may be hydrolized by specific ferments in all tissues of a plant where a sufficient supply of water is available. This may also occur with cane sugar and perhaps with other soluble carbohydrates, although the change in these cases is more readily effected in the leaves, as is shown by a lower content of sugar in the sap of leaves than in that of other tissues as well as by the rapid disappearance of cane sugar from beets, from bulbs, and from the sap of deciduous trees, when leaves appear in spring. The increase of cane sugar in all sugar producing plants as maturity approaches and leaves become less active, is further evidence of this trend.

A most remarkable thing about plant metabolism is that none of the organic excreted products of the cells, except a small part of the carbon dioxide and a few volatile oils and ethers manifested by a characteristic odor, are lost to a plant, after photosynthesis is established.

#### WATER PRODUCED DURING THE RIPENING OF FRUIT

Some of the best illustrations of the changes induced by respiration, in the character of vegetable tissues, are found in the ripening of fruits, especially such as apples, pears, plums, and other varieties in which the final ripening changes may be normally completed after the fruit is removed from the tree. During the period of growth, the stems of such fruits are firmly attached to the parent branch, but as maturity approaches, the cellular structure in a portion of the stem changes, some of the cells being absorbed so that a slight strain may separate the fruit from the tree. This stage is usually reached while the fruit is still hard and green, and before it has reached an edible condition. It is doubtful if the fruit receives much water or other nutrients from the tree, after this time, although its cells are still active and respiration continues at a rapid rate. Picking the fruit does not stop the normal ripening changes, which proceed fully as rapidly after removal from the tree as before.

These changes are manifested by an absorption of oxygen, a corresponding evolution of carbon dioxide, and a marked increase in the mellowness and succulence of the fruit.

During ripening, there is a constant loss of organic matter through oxidation, which naturally results in the production of considerable metabolic water, but the effect of this upon the succulence of the ripe fruit is to a considerable extent offset by hydrolytic reactions in which water is fixed in organic combination. The tissues of green fruits nearly always contain starch, pectous substances and various organic acids which mostly disappear during ripening, being converted into soluble carbohydrates and other compounds in which the elements of water are higher than in the original form. Nearly all of the changes occurring in the ripening of fruit, except those resulting from direct oxidation, are hydrolytic in character and cause liquid water to disappear. The water thus fixed in organic combination may in some cases exceed the metabolic water resulting from oxidation and still leave the ripe fruit far more succulent than the green fruit, because more of the organic matter of the ripe fruit is dissolved in the fruit juices.

It is probable, also, that some water is fixed by photosynthesis, when green fruits are exposed to light, even after the fruits are removed from the tree. In addition to the withdrawal of water for these purposes, there is a continual loss of water into the surrounding air, even when this is in a saturated condition, since the carbon dioxide evolved from the wet tissues must be saturated with water.

In spite of the water withdrawn from a fruit in these ways, during the ripening, there is, under ordinary conditions, a continual increase in the percentage of water found. This increase is partly due to loss of organic matter, through oxidation, and partly to the substitution of water for a portion of the organic matter that has disappeared. It often happens, when fruit is stored under conditions which retard evaporation, that the metabolic water produced by oxidation, is more than sufficient to replace that lost by evaporation and that fixed in organic combination, in which case, the absolute amount, as well as the percentage of water in the ripened fruit, exceeds that present in the green fruit when it was picked.



## RELATIVE WATER CONTENT OF GREEN AND RIPE FRUIT

During the past four or five years, as opportunity offered, determinations of water have been made in various kinds of fruit, at different stages of maturity, for the purpose of ascertaining the amount and direction of these changes. All of the fruits, except the apples and plums, were purchased in the markets and had been picked some time when observations commenced. The apples and plums were seedling varieties grown on the station grounds. The fruits selected for each experiment were apparently in the same stage of maturity, but with the exception of apples and plums may have been grown on different trees, and possibly in different orchards, under conditions which materially affected the initial water content.

It has been assumed in each case that the water content of the fruit set aside to ripen was the same as that found in the green fruit, at the date when this was examined, and that the difference in the water content of the green and ripe fruit is the result of ripening changes. There is some error by this assumption, but since in nearly every case the results are either averages of several determinations, or are derived from composite samples taken from different specimens, it is believed that they are typical of the changes that occur in the normal ripening of fruit.

The water content was determined, in every case by drying to constant weight in a steam oven at approximately 97°C. The loss in weight was calculated on the original weight of the green fruit. The results are given in Table XV.

The most obvious change, during the ripening of fruit, shown in Table XV, is the increase in the percentage of water; a change that always occurs when excessive evaporation of water is prevented, whether fruit is left hanging upon the tree, or is picked.

There is a general impression, among fruit growers, that pears picked as soon as the fruit will separate from the branch without breaking the stem, and stored in a dark place to ripen are sweeter and more succulent than fruit left to ripen upon the tree. This is easy to understand, since the fruit receives little water from the tree after it is mature and the connecting tissues of the stem are being absorbed. It is even doubtful if sufficient

TABLE XV. WATER CONTENT OF GREEN AND RIPE FRUIT

Sample No.	Variety	GREEN FRUIT		RIPE FRUIT		Per cent loss in weight
		Date	Per cent water	Date	Per cent water	
1	Bartlett pear.....	Aug. 19	81.64	Aug. 26	82.57	6.08
2	Bartlett pear.....	Sept. 25	85.73	Sept. 30	86.23	.....
2	Bartlett pear.....	Sept. 25	86.73	Oct. 4	87.62	2.06
3	Seckel pear.....	Aug. 31	79.76	Sept. 18	80.36	.....
4	Seckel pear.....	Sept. 9	77.01	Sept. 9	78.02	.....
5	Seckel pear.....	Sept. 22	76.96	Oct. 1	78.63	0.54
6	Seckel pear.....	Oct. 2	80.71	Oct. 15	81.47	0.38
6	Seckel pear.....	Oct. 2	80.71	Oct. 20	81.03	1.33
6	Seckel pear.....	Oct. 2	80.71	Oct. 22	81.88	2.98
7	Winter Nellis pear.....	Nov. 23	76.52	Dec. 14	77.95	0.90
8	Winter Nellis pear.....	Nov. 25	75.73	Dec. 8	77.57	4.24
9	Apples.....	Aug. 18	81.29	Aug. 31	83.88	5.50
10	Crab apples.....	Aug. 18	82.10	Aug. 31	79.86	16.16
11	Plums.....	Sept. 26	81.43	Oct. 5	82.12	8.69
11	Plums.....	Sept. 26	81.43	Oct. 6	82.32	5.98
11	Plums.....	Sept. 26	81.43	Oct. 6	82.85	1.41
12	Plums.....	Sept. 26	83.07	Oct. 5	83.21	8.80
12	Plums.....	Sept. 26	83.07	Oct. 6	83.65	5.95
13	Plums.....	Sept. 26	79.67	Oct. 13	78.69	11.54
13	Plums.....	Sept. 26	79.67	Oct. 21	79.76	8.75
14	Plums.....	Sept. 3	87.00	Sept. 7	87.89	1.40
14	Plums.....	Sept. 3	87.00	Sept. 13	89.49	4.36
15	Plums.....	Sept. 3	80.67	Sept. 13	82.10	6.12
15	Plums.....	Sept. 3	80.67	Sept. 6	80.80	.....
15	Plums.....	Sept. 3	80.67	Sept. 14	81.75	.....
15	Plums.....	Sept. 3	80.67	Sept. 18	81.90	.....
15	Plums.....	Sept. 3	80.67	Sept. 24	83.84	.....
16	Plums.....	Sept. 18	81.90	Sept. 20	82.68	0.96
16	Plums.....	Sept. 18	81.90	Sept. 22	83.60	2.42
16	Plums.....	Sept. 18	81.90	Sept. 24	83.82	4.82
17	Plums.....	Sept. 24	83.84	Sept. 29	84.96	3.24
17	Plums.....	Sept. 24	83.84	Oct. 4	84.84	9.93
18	Plums, pulp only, same as No. 14.....	.....	.....	Sept. 7	90.49	.....
18	Plums, pulp only, same as No. 14.....	.....	.....	Sept. 13	91.55	.....
19	Pulp from No. 15.....	Sept. 6	86.06	Sept. 13	86.35	.....
19	Pulp from No. 15.....	Sept. 6	86.06	Sept. 14	86.75	.....
19	Pulp from No. 15.....	Sept. 6	86.06	Sept. 18	86.72	.....
19	Pulp from No. 15.....	Sept. 6	86.06	Sept. 20	87.37	.....
19	Pulp from No. 15.....	Sept. 6	86.06	Sept. 22	87.69	.....
19	Pulp from No. 15.....	Sept. 6	86.06	Sept. 24	88.25	.....
19	Pulp from No. 15.....	Sept. 6	86.06	Sept. 24	88.37	.....
19	Pulp from No. 15.....	Sept. 6	86.06	Sept. 29	90.81	.....
19	Pulp from No. 15.....	Sept. 6	86.06	Oct. 4	88.50	.....
20	Japanese persimmon.....	Oct. 28	86.06	Nov. 6	75.17	4.22
20	Japanese persimmon.....	Oct. 28	86.06	Nov. 19	79.68	0.81
21	Japanese persimmon.....	Oct. 30	76.75	Nov. 18	77.43	1.11
21	Japanese persimmon.....	Oct. 30	86.06	Nov. 16	77.38	0.79
22	Mushroom, (coprinus).....	Sept. 13	95.16	Sept. 14	95.51	2.29
23	Mushroom, (coprinus).....	Sept. 21	93.13	Sept. 22	94.42	3.42

water is derived from the tree during this period to replace that lost by evaporation which, on account of exposure to free circulation of air, is much greater than with fruit stored in bulk. It is probable that so long as green fruit is exposed to light, photosynthesis with the production of a carbohydrate from carbon dioxide absorbed, and water abstracted from the fruit, continues to occur and thus diminish the percentage of water in fruit ripened upon the tree. It is not unlikely that the mealy, granular condition often found in such fruit is partly due to this cause. No doubt such changes also take place after fruit is gathered, if it is exposed freely to light, and tend to make the fruit less succulent than when it is stored in darkness.

Succulence of fruit depends, not only on the ratio between the solids and water, but on the nature of the solids. A fruit containing much of such solids as sugar and organic acids that are soluble, appears to be much more succulent than a fruit in which the organic matter is chiefly in the form of cellulose, starch, and insoluble pectous substances, which usually predominate in green fruit. During the ripening changes these insoluble substances mostly disappear being replaced by soluble carbohydrates, a change which makes the water content appear to increase, even when an actual decrease has occurred.

The loss in weight, through respiration and transpiration, during ripening, is not necessarily associated with a shrinkage in volume since the products of oxidation of organic matter, aside from carbon dioxide, are usually of about the same and often of greater volume than the substances that have disappeared; this is especially true when fruits are ripened in a nearly saturated air, where evaporation is small. It is chiefly for this reason that gathered fruits sweat when placed in large piles or in close receptacles, the exudation being greatly increased by internal pressure induced by an increase in the volume of the products formed.

With some fruits that have a tender skin, the internal pressure is manifested by exudations of juice upon the surface, or by cracking of the skin. It frequently happens during damp weather that plums crack upon the tree. That this is not caused by water derived from the tree is evident, since sound fruit placed in a covered vessel in which the air is nearly saturated with moisture cracks fully as much as fruit left upon the tree.

The influence of free evaporation and possibly of photosynthesis is clearly shown, in the case of plums, in experiment 15 of Table XV where the first sample, picked September 3, contained 80.67 per cent water. The ripe sample analyzed September 13 was picked the same day as the first and allowed to ripen in a covered vessel in the laboratory. The ripe sample contained 82.10 per cent water, an increase of 1.43 per cent in ten days. All of the other samples were picked on the date when the analyses were made. In every case there was found a higher percentage of water than on the first date but with the exception of the last sample picked September 24 when the fruit was overripe and past its best condition, none of them contained so high a percentage of water as did the sample ripened off the tree.

#### INTRAMOLECULAR RESPIRATION OF FRUITS

The fleshy tissues of fruit consist largely of living cells which continue to be active for some time after the fruit is mature, even though it may be separated from the parent plant. The ripening changes of the fruit, by which its texture is broken down, its succulence increased, and its characteristic flavors developed, are all vital processes depending upon these cells and anything that interferes with their activity affects the quality of the ripened fruit. If, at any stage of ripening, the cells are all killed, the process is suspended, except for slight changes induced by specific enzymes present in the fruit. If the cells are killed by heat, or by poisons that suspend the activity of enzymes, the fruit remains indefinitely in the same condition. All methods employed to preserve fruit involve this principle as well as the exclusion or inhibition of the organisms of decay.

Energy required for maintaining the activity of fruit cells arises from the oxidation of organic nutrients in the same way as in other tissues of a plant. Oxygen required for this may be derived from the air by direct respiration, as in normal ripening, or energy may be derived from organic nutrients containing oxygen, by intramolecular respiration. The amount of nutrients required in the latter case, to supply the necessary energy, is greater than when free oxygen is available, since the substances formed in intramolecular respiration are not com-

pletely oxidized. Among these products are various organic acids, alcohols, aldehydes, and esters, which are more or less toxic to living cells, especially when abundant. Moreover these products accumulate in the fruit rapidly, since no means are provided for their removal and restoration into suitable nutrients, as is the case in a growing plant through photosynthesis. For these reasons, the life of cells composing the tissues of fruit, from which free oxygen is excluded, is relatively short, and the resulting texture and flavor of the fruit are widely different from that developed when the fruit is ripened in contact with air. This has been illustrated in several experiments with apples and pears, during the past ten years. Both of these fruits, if picked at early maturity and placed in oxygen-free air, continue to evolve carbon dioxide for three or four weeks, during which time the general appearance of the fruit remains practically unchanged. Its cells, however, die and it acquires an unnatural odor and taste suggestive of corn silage. When exposed to air, these dead fruits dry quickly and a cut surface remains white indefinitely, in sharp contrast with the behavior of fruits of the same variety that have been ripened in normal air. The water content of fruit deprived of free oxygen increases considerably during the first few weeks but, because of its peculiar texture, the succulence of the fruit appears to be no further advanced than in the green fruit, at the beginning.

If oxygen be excluded from these fruits for a longer period then three or four weeks, the color of the skin gradually changes to dull brown, the cut surface also becomes dark and appears to be water soaked. Drops of exuded water appear upon the surface, and sometimes water collects in the bottom of the vessel. The acidity of the fruit increases and the acid odor is more marked. The texture remains unchanged. After eight or ten months, no further change is apparent, so long as free oxygen is excluded.

It seems probable that all vital activity of the fruit cells ceases when no more carbon dioxide is evolved, since this indicates the total suspension of intramolecular respiration. In no case has this continued, with either apples or pears, beyond thirty days. The changes that occur after this time appear to be caused by enzymes present in the fruits, since the action of these is not dependent upon vital processes. In a few cases,

anaerobic organisms have attacked the fruit from which oxygen was excluded, causing it to decay rapidly, but changes of this nature are easily distinguished from those induced by active cells of the fruit.

No difference has been noticed in the final results when oxygen has been replaced by hydrogen, nitrogen, carbon dioxide, or by the residual gases when the oxygen in the containing vessel is absorbed by the direct respiration of the fruit itself. In the latter case, active intramolecular respiration has been delayed until the free oxygen was exhausted. The same results were obtained when oxygen was excluded by immersing the fruit in cotton seed oil. This method has some advantages, since it prevents the passage of anaerobic organisms from one specimen to another.

Table XVI shows the loss in weight and the water content of Seckel pears that were kept in carbon dioxide.

TABLE XVI. WATER CONTENT OF PEARS IN CARBON DIOXIDE

Water content and loss in weight of Seckel pears kept in carbon dioxide.

Months exposed	Per cent water content.	Per cent loss in weight.	Per cent of dry matter referred to fresh fruit
0.....	80.71	.....	19.29
2.....	85.38	2.37	14.27
13.....	85.84	5.87	13.33

Changes in fruits, when deprived of free oxygen are analogous to those found in similar experiments made in cooperation with Dr. Russell,<sup>12</sup> with succulent plants, to determine the causes operative in the production of silage.

The succulent tissues of growing plants of all kinds behave in an analogous manner, when deprived of free oxygen, the only difference arising from the relative numbers of active cells present and the amount of suitable nutrients available for the support of intramolecular respiration. In young succulent tissues, in which the cells are active and the nutrients mostly in a soluble form, that is directly available, the production of acid products is rapid and the life of the cells relatively short. Plants in this condition are not suited for silage, since they produce an

<sup>12</sup> 17th Rpt. Wis. Exp. Sta., 1900, p. 123.

18th Rpt. Wis. Exp. Sta., 1901, p. 177.

excessively wet, acid product that has little nutritive value. On the other hand, the same variety of plant, in a more mature condition, with comparatively few active cells, and with most of the nutrients in an insoluble form, makes an excellent silage containing a minimum amount of water and acid, and having a high nutritive value. The prevailing practice, among the better farmers is fully in accord with these principles.

#### MOVEMENT OF WATER IN PLANTS

No question has been raised by any one regarding the primary source of water used by plants, but there has been, and still is, great difference of opinion regarding the method of its distribution in the plant. A full account of the experimental work bearing upon the problem has been given by Pfeffer,<sup>13</sup> also by H. Marshall Ward.<sup>14</sup> Extended and careful observations regarding phenomena associated with the flow and pressure of sap in the maple were made by Jones, Edson and Morse.<sup>15</sup> A more recent resume of the subject, especially in relation to the action of living cells upon transpiration and sap flow has been made by Overton.<sup>16</sup>

It is conceded by all that water, absorbed by the root hairs, passes up through the sap wood, which abounds in active cells, to the leaves, where it is either used in photosynthesis for the production of organic matter, or passes into the air by transpiration. Little if any water is transmitted through the heart wood, which contains no living protoplasm. A tree may continue to grow and maintain all of its normal functions, for an indefinite period, after the heart wood is wholly destroyed by fungus disease. This suggests a close causal relation between the vital processes occurring in the sap wood and the transmission of water to the leaves.

One of the most characteristic functions of living protoplasm is respiration which is always manifested by an evolution of carbon dioxide. In most cases it is also associated with a direct absorption of free oxygen although, if suitable nutrients are continually supplied and the waste products removed as fast

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<sup>13</sup> Physiology of Plants.

<sup>14</sup> Timber and Some of Its Diseases.

<sup>15</sup> Bulletin 103. Vt. Exp. Sta.

<sup>16</sup> Studies on the Relation of the Living Cell to the Transpiration and Sap Flow in *Cyperus*, Bot. Gaz. Jan. and Feb. 1911.

as they are formed, respiration may for some time be wholly intramolecular, no free oxygen being removed. The cells of living tissue are continually respiring by one or both of these methods and in consequence there is a constant production of carbon dioxide, water, and certain soluble organic compounds in these tissues. The corky tissues of the bark interpose an almost impervious barrier to the direct escape of the carbon dioxide formed. On the other hand, the sap wood with its porous structure, through which a current of water is continually passing, provides an efficient means for its removal, since carbon dioxide, especially when subjected to pressure, is readily soluble in water and is either carried by it in solution, or passes by osmosis from one vessel to another to the leaves where it is restored by photosynthesis into nutrient materials available for the support of respiration. The amount of carbon dioxide in the gases dissolved in the sap of growing plants, which amounts to 100 to 500 times that in normal air, and from ten to fifty times that in soil air, confirms the view that this is the usual path by which the gaseous products of respiration are removed.

The presence of organic acids, sugars of various kinds, and other products formed by protoplasmic activity, in the sap is further evidence of a direct transfer of soluble matter from the active cells to the vessels in which water is carried to the leaves, since the water absorbed by the roots contains none of these substances.

For the most part, organic substances found in circulating sap are products of intramolecular respiration and do not serve as direct nutrients for growing cells. Some of these products may be converted into suitable nutrients by specific enzymes in any part of a plant where direct respiration occurs, others are made available as nutrients, only by photosynthesis, chiefly in the chlorophyll bearing cells of the leaves.

It is highly probable that the evolution of carbon dioxide during respiration, is one of the chief factors affecting sap flow and pressure in growing plants. Sachs advanced the idea that the change in volume of gases in plant tissues, caused by variation of temperature is the cause of changes in sap pressure, and through its intermittent action, of sap movement. This view is discussed by Jones, Edson and Morse<sup>17</sup> in its relation to

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<sup>17</sup> Loc. cit.



phenomena observed in the maple and other trees, in which pressures occur many times greater than can be accounted for by simple expansion at observed temperatures, and while admitting that this factor has some influence, they consider it to be of minor importance.

There is, however, one phase of this question that has, apparently been overlooked and that is the influence which temperature and pressure have upon the solubility of carbon dioxide in water. The behavior of carbon dioxide, in this respect, differs greatly from that of either oxygen or nitrogen, the gases with which it is associated in the air. For comparison, the solubilities of nitrogen, oxygen and carbon dioxide, in water, are given in Table XVII. The values for each gas are taken from A. Seidell's tables of solubilities.

TABLE XVII. SOLUBILITY OF NITROGEN, OXYGEN, CARBON DIOXIDE  
Gases dissolved in water at different temperatures. The weights and volumes are for one c. c. of water

TEMPERATURE C°	NITROGEN		OXYGEN		CARBON DIOXIDE	
	Wt. gms.	Vol. c. c.	Wt. gms.	Vol. c. c.	Wt. gms.	Vol. c. c.
0.....	.00239	.0235	.00685	.0489	.335	1.713
5.....	.00259	.0208	.00607	.0429	.277	1.424
10.....	.00230	.0186	.00537	.0380	.231	1.194
15.....	.00208	.0179	.00480	.0342	.197	1.019
20.....	.00189	.0164	.00434	.0310	.169	0.878
25.....	.00174	.0150	.00393	.0283	.145	.759
30.....	.00161	.0138	.00359	.0261	.126	.665

It appears from Table XVII that, at 0°C., water dissolves 1.7 times its volume of carbon dioxide which is thirty-five times greater than the volume of oxygen and more than seventy times greater than the volume of nitrogen dissolved at the same temperature. It is also evident that the solubility of carbon dioxide increases more rapidly as temperature falls than either of the other gases, the increase in solubility for one degree change in temperature between 0° and 5°C. being more than the total solubility of either nitrogen or oxygen at 0°C. It is obvious that while it may be permissible to ignore the influence of solubility upon the pressures of both oxygen and nitrogen, in the presence of water, this cannot be done without serious error if a large proportion of carbon dioxide is present, as is always the case in the gas in plant tissues, especially when photosynthe-

sis is suspended, at which times, maximum pressures usually occur.

No data are available regarding the solubility of carbon dioxide at temperatures below  $0^{\circ}\text{C}$ ., but since its solubility increases as this point is approached, it is probable that the solubility increases still more rapidly as the temperature falls below freezing. This has a direct bearing upon the problem since the sap of plants always contains substances (chiefly sugars and organic acids) in solution which naturally lower its freezing point. Moreover, as freezing proceeds the concentration of the sap increases causing the freezing point to be continually lowered. It seems probable therefore, that some concentrated sap always remains liquid at all temperatures which prevail during the season when sap flows or when sap pressure is observed. The negative pressure observed at low temperatures, as well as the positive pressure that follows when the temperature rises above freezing, are in strict harmony with the view that carbon dioxide is dissolved at the lower temperature and liberated from solution when the temperature is raised.

Sap pressure in maple and certain other trees is most pronounced in early spring, before the appearance of leaves, when transpiration is slow and when the diurnal range in temperature is wide and when respiration is stimulated by an abundance of suitable nutrients, in soluble form, that have accumulated during a prolonged dormant period. The conditions are therefore most favorable for the production of a maximum pressure during the day and of a minimum, perhaps a negative pressure during the colder nights. Among the most important factors that contribute to these results is the influence of temperature upon the evolution and solubility of carbon dioxide and this alone is believed to be sufficient to explain sap pressure and the bleeding of the maple and other plants which exhibit these phenomena in early spring, before photosynthesis is established. It certainly is not dependent on water derived directly from roots, for trees cut in early winter before any sap pressure has developed bleed copiously in the warm days of spring and under favorable conditions the buds swell and may open. Nursery stock kept in storage during the winter under conditions that allow no absorption of water through the roots, put forth leaves and blossoms in the spring. The same is true of most bulbs and tubers which send forth long sprouts with no external water supply.

As the season advances, the temperature rises and becomes more uniform. These conditions favor a more rapid respiration with a greater evolution of carbon dioxide and if other conditions remained the same, an enormous pressure would necessarily result from the greatly increased vital activity. Coincident with these changes leaves develop, photosynthesis is established, transpiration increases, and the pressure is relieved. A large part of the carbon dioxide resulting from respiration, and much water, are utilized during photosynthesis, in the production of organic nutrients and a large amount of water escapes through the leaves in transpiration. These functions not only prevent excessive pressure but usually induce a negative pressure during the growing season.

During the growth of an annual plant 200 to 400 pounds water are absorbed by roots and transpired, for each pound of dry matter contained in the mature plant. Since the elements of water comprise but little more than half the weight of the dry matter of plants, this amount is greatly in excess of the actual needs for growth, but in addition to supplying material for building tissue, water is the medium by which the waste products of respiration are removed from the cells and carried to the leaves for restoration into nutrients that may be used again. A large amount of water is required for this function because the concentration of the excreted products must be kept low to avoid injury, and in the case of carbon dioxide, which is but slightly soluble, to insure its solution and prevent its waste.

During the day, when photosynthesis is active, the proportion of carbon dioxide in the gas of plants is lower than in early morning, after the plant has been in darkness for several hours. This indicates that the carbon dioxide liberated by respiration during the shaded period has not escaped through the leaves and bark as rapidly as it has been formed, but has been dissolved for the most part, in the plant sap, and held in reserve for resynthesis into available nutrients when day-light returns. This principle applies also to other soluble products of respiration found in the circulating sap of plants, most of which are returned to the leaves and converted into suitable nutrients to be used again. The carbon dioxide evolved by a plant in darkness is not therefore an accurate measure of its vital activity, unless the

shaded period is quite prolonged, since a large part of the carbon dioxide formed under these conditions is held in solution in the sap, and finally converted into suitable nutrients for the plant.

This retention of carbon dioxide and other products of respiration, when a plant is shaded, serves a most important purpose in the conservation of carbohydrate nutrients, which must otherwise be lost, since during photosynthesis these excreted products are restored to their original form and once more made available for the building of tissue and for maintaining the vital forces. By this means the waste of nutrients through respiration is greatly reduced and the rate of growth correspondingly increased. In this respect chlorophyll producing plants are far more economical in the utilization of nutrients, than are saprophytic plants or animals, which have no provision for restoring products of respiration into nutrients that may be used again for maintenance or growth, and which must expend considerable energy in the elimination of these useless and often poisonous excretions.

The behavior of girdled trees and plants shows that water absorbed by roots is transferred in some way, through the woody tissue to the leaves and that very little movement of water, in this direction, occurs through the more active cells of the cambium and cortex. No doubt these derive some water, by osmosis, directly from the channels through which the upward current of water is carried, but most of the water in these tissues appears to pass first to the leaves and from there in organic combination, to the growing cells, in all parts of the plant.

It is well established that organic nutrients are synthesized in the leaves and then distributed. The course taken by these nutrients cannot be through the same channels by which water is brought from the roots to the leaves, for the current in these vessels is always in the opposite direction, and during active transpiration, is far too rapid for osmosis to overcome. Since there is no free current of water through the growing cells these nutrients must pass from one active cell to another, by osmosis, from the leaves to the remotest part of a plant. Every condition, in a growing plant, is favorable for such a movement, the concentration of nutrients being naturally higher in the leaves, where nutrients are elaborated, and where evaporation is most rapid, than in neighboring cells where no synthesis oc-

curs and from which respiration is constantly removing a portion of the dissolved substance and replacing it in part, with metabolic water. Moreover this condition holds for each succeeding cell wherever active protoplasm exists. There is of course a transfer of water, by osmosis, in the opposite direction, from the growing cells towards the leaves but this is more than compensated by the production of metabolic water through the oxidation and dehydration of organic nutrients. When they start from the leaf, carbohydrate nutrients are in the form of dextrose or similar substance containing a large proportion of the elements of water, but, as they proceed, a portion is completely oxidized by the respiration of each cell and all of its potential water set free; a portion is deposited as cellulose or starch containing less of the elements of water than the original nutrient; and still other portions are transferred, together with various products of respiration, into the current of water that flows constantly through the woody tissues from the roots, and are carried back to the leaves from which point the cycle is repeated, so long as water is supplied to the roots and conditions are favorable for the synthesis of organic nutrients in the leaves.

One of the most important results of this cycle is the liberation of metabolic water in all growing cells. It is to this that the high water content of such tissue is chiefly due and it is this more than any other factor, which determines the direction and intensity of the osmotic movement of nutrients from the leaves towards the respiring cells. Imbibed water, that is water from an external source, which contains no nutrients that are directly available for supporting respiration, tends to withdraw nutrients from any cell with which it is in contact, whereas metabolic water, produced by the oxidation or dehydration of organic nutrients within the cell walls, tends in the opposite direction and attracts nutrients to the points where they are most needed. The first leads to the starvation and death of a cell, the second to its development and growth.

The respiring cells of a mature plant are nourished in the same way as those in the sprouts of a germinating seed, before photosynthesis is established. In both cases, nutrients are brought to the cells by osmosis, where they are oxidized and dehydrated by respiration, in the same manner, giving rise to products of the same general nature. The seedling, however,

at this stage of development, depends for its growth solely upon a limited store of nutrients that is being constantly depleted, since it is incapable of acquiring organic nutrients from an external source, while the products of respiration are all wasted. Photosynthesis changes this condition, each leaf becoming a laboratory in which carbon dioxide from external sources together with a large part of the carbon dioxide resulting from respiration, and practically all other excreted products from cells in all parts of the plant, are elaborated into available food materials. From this time on, new sources of supply distributed over the plant add to, and finally wholly replace the reserve stored in the seed. There is thus provided a surplus, and the distance through which nutrients must be carried to meet the demands of respiring cells, is greatly diminished. In this way energy is conserved to a plant and its rate of growth greatly augmented.

#### WATER PRODUCED IN ANIMAL METABOLISM

The energy required for maintaining the vital functions of animal cells is derived from the oxidation of nutrients, through the respiration of protoplasm, in a manner entirely analogous to that which occurs in vegetable cells. In both cases, the nutrients are oxidized within the cell wall, where all of the resulting products are set free. These products, which consist principally of carbon dioxide and water, are gradually removed from the cell by osmosis and diffusion. In consequence of these changes, there is in every living cell, whether it be vegetable or animal, a continual replacement of a part of the organic nutrients by water thus maintaining the nutrient solution, within the cell wall, at a lower concentration than in the fluids which distribute the nutrients to the tissues. This difference in concentration insures a constant movement of nutrients, by osmosis, towards the points where they are needed, thus providing for the growth and maintenance of tissues, so long as suitable nutrients are available for the purpose.

In this respect, no difference is apparent between the vegetable and animal kingdoms. The rate of oxidation is, however, necessarily far more rapid with animals than with plants, in order that sufficient energy may be provided for maintaining muscular activity. This demands a more abundant supply of nutrients,

and results in the production of a larger quantity of metabolic waste materials, per unit of weight, with animals than with plants.

Another and more important difference is the inability of animals to resynthesize the organic waste products of respiration into substances that may be again utilized as nutrients. Most metabolic products, except water, if allowed to accumulate, exert a toxic action upon animal cells, and must therefore be eliminated from the organism as fast as they are formed. This is especially the case with the soluble products arising from protein metabolism. With most animals, these nitrogenous products are excreted in solution through the kidneys, chiefly as urea, but birds, reptiles, and all insects excrete most of the nitrogenous waste matter as uric acid, or its ammonia salt, which being practically insoluble in the body fluids, is voided in a solid condition.

The organic nutrients utilized by animals are all derived from external sources and no water need be consumed for their synthesis, as is the case with plants; for this reason, all of the metabolic water arising from the oxidation of nutrients by an animal, is available for other vital processes, and for replacing evaporated and excreted water. Moreover, under similar atmospheric conditions, the loss of water by evaporation is far greater per unit of weight with plants than with animals because of the enormous leaf surface exposed directly to currents of air, and because in the case of animals the surface is usually protected from a rapid change in hygroscopic conditions by a covering of hair, wool, or feathers, and in the case of some insects in the larval state, by a silky envelope. For these reasons, a much larger proportion of the water required for the vital functions of animals is supplied by metabolic changes in the food and tissues than is the case with plants. With many animals, nearly all of the water required is derived from the oxidation of organic nutrients and there are good reasons for believing that metabolic water would be sufficient for all animal needs, were it not for the elimination of poisonous nitrogenous products formed in protein metabolism.

The cell walls of animal tissues are mostly protein in character while those of vegetable tissue are composed of carbohydrates. This difference in composition necessitates a much larger pro-

portion of nitrogen in the food of animals than in that of plants, and also makes the poisonous products of metabolism, which must be removed, very large, since animals are provided with no means for regenerating such products into nutrients, as is brought about in plants by photosynthesis.

The need for water is much less for animals that excrete uric acid than for those that excrete urea, since uric acid, being practically insoluble in the body fluids, is not so poisonous as urea and is voided solid with a minimum loss of water. Many animals that excrete uric acid instead of urea never have access to water and subsist in every stage of their development upon air dried food which usually contains less than 10 per cent water. The most striking illustrations of this kind are found among insects such as the clothes moths, the grain weevils, the dry wood borers, the bee moths, etc. The larvae of these insects contain a high per cent of water, and the mature forms, in spite of the development of wings which are relatively dry, rarely contain less than 50 per cent water. It is fair to assume that all of this water is metabolic, since the insects never have direct access to water and it is extremely doubtful if the free water in their food is sufficient to replace the water lost through respiration, by evaporation from the surface, and as a part of the excreta.

It has been suggested that the tissues of these insects are hygroscopic and absorb sufficient water from the surrounding air to supply all of their needs, but when killed the bodies of these insects dry quickly upon exposure to ordinary air, a change that could not occur if the substances that compose their tissues were inherently hygroscopic. In this respect the bodies of such insects do not appear to differ materially from other animal tissues. The only remaining source of water is the metabolic changes in the food and tissues which appears to be amply sufficient for all purposes, if enough suitable food is available.

For several years observations have been made upon insects, especially those capable of subsisting upon air-dry materials, to determine the minimum water requirement. The most important of these observations are here given in detail.

#### THE CLOTHES MOTH (*Tinea Pellionella* Linn.)

In 1905 several clothes moths were placed in a desiccator the air of which was dried with sulphuric acid. There were



placed in the same vessel pieces of woollen cloth that had been previously dried at a temperature approximating 97° C. The millers remained active for several days, none dying until the tenth day, but all were dead by the fourteenth day. The mature insects subsist entirely upon nutrients consumed and stored in the larval state, and since they are quite active, live but a short time under the most favorable conditions. It is doubtful if their life was materially curtailed by exposure to dry air.

Before death, these millers deposited many eggs upon the dry cloth, most of which hatched, the young larvae being seen crawling over the cloth. The larvae varied in size, and particles of excrement were found in the vessel indicating that the larvae had consumed and assimilated some of the dry food. No live larvae were found after the twentieth day following the confinement of the millers. It is not known how long any of the larvae lived, under these conditions; it is certain, however, that the millers laid eggs which hatched, and that the young larvae lived several days, upon dry food, in an atmosphere practically free from moisture. When the desiccator was opened, at the end of the experiment, the confined air had a distinct odor of sulphur dioxide and it was thought that this, rather than the extreme dryness, might have been the cause of death. To determine this, a piece of dry woollen cloth was placed in a cage containing moth millers and left there several days, until eggs were deposited upon it; when these eggs began to hatch, the cloth was removed to a ventilated desiccator containing calcium chloride. Live larvae were seen upon the cloth every day for two weeks, but none were discovered after this time. On the nineteenth day the cloth was removed and no live larvae found.

It appears from these experiments, that young larvae of this species of insect, cannot long survive the strain induced by exposure to dry air. It has been suggested that the larvae in the tests lived until the first molting period, at which time all insects are known to be susceptible to adverse conditions of any kind. This is uncertain, since the length of time which any one larva lived is unknown. Be that as it may, it seems far more probable that excessive evaporation from the small, thin skinned larvae, rather than the dry condition of the food provided, was the direct cause of death, in these cases. The rapid development of the larvae, in the open air, while receiving no food containing more than 5 per cent water, supports this view. I have

no doubt that these larvae would grow to maturity, and complete their life cycle an indefinite number of times, while receiving dry food, if this could be supplied under average atmospheric conditions. The hygroscopic nature of hair, wool, etc, which constitute the natural food of these insects, makes an experiment of this kind impracticable.

At another time, a mink fur collar slightly infested with moths, in the larval stage, was placed in a breeding cage for the insects to develop. The fur was in a paper sack and was not removed when it was placed in the cage, although the sack was left open to allow the moths to fly around when they emerged from the cocoons. A sample of the fur was removed for a water determination, the result of which is given later. But few millers appeared from the first brood and these were allowed to lay eggs without being disturbed. Three months after the fur was put in the cage, it contained many larvae that a month later appeared to be full grown. The mature larvae were active. Some of these were removed for analysis, others were transferred to another cage containing wool feathers, a piece of astrakhan fur, and woolen cloth, the water contents of which were known.

Some time after larvae ceased to crawl around, in the first cage, it was opened and carefully examined but no live larvae were found. It was then discovered that every particle of fur had been consumed, leaving the skin clean and white. The silk trimmings had not been touched. It seems probable that the larvae left in the cage were starved to death, and that the active movements observed were made in search of food and not to find a secure place to spin a cocoon, as was at first supposed. The larvae that were transferred to a cage containing different food materials completed their life cycle and passed through several generations during the next six years, but the number of insects observed at any one time was small compared to those grown upon the mink fur. In the last cage the insects preferred the astrakhan fur to other food materials present; next to this the wool, no difference being observed between the washed and unwashed samples. The feathers were scarcely touched.

Water determinations were made in the larvae of the clothes moth at three different times; the results are given in Table XVIII, together with the water content of the air dry material

upon which the larvae fed while passing through this stage of development.

TABLE XVIII. WATER IN MOTHS AND IN THEIR FOOD  
Water content of larvae of clothes moths, and of materials upon which they feed

WATER CONTENT OF LARVAE		WATER CONTENT OF FOOD MATERIAL	
Size	Per cent water	Material	Per cent water
Full grown.....	57.66	Woolen cloth.....	6.11
Full grown.....	57.88	Unwashed wool.....	7.56
Medium.....	59.83	Washed wool.....	3.66
		Hair from fur.....	9.08

The great difference in water content between the food materials and the larvae leaves little doubt that they depend chiefly upon metabolic water for all of their vital processes. It is doubtful if the water in the food is, in any case sufficient to replace that lost in normal respiration. Evaporation from the skin is small in any case and in the mature larvae it is greatly reduced by an envelope consisting of particles of the food material bound together by a silken web. It seems likely that the fur supplied more favorable conditions for development than did the other food materials, because its water content was higher, and that this is the reason why more larvae appeared in this than in wool. There were, however, some individuals that passed through both the larval and pupal stages in all of the materials. It is interesting to note that the smaller, growing larvae, in which the most active metabolism occurred, contained the highest proportion of water.

Much excrement from the larvae was found in the paper sack enclosing the mink fur collar. This was examined by Prof. E. V. McCollum and a large part of its nitrogenous constituents determined. Of the total weight of the dry samples 26.66 per cent was nitrogen; 10.22 per cent of the nitrogen was voided as ammonia, 47.29 per cent as uric acid, 17.57 per cent as urea, 10 per cent as creatin and creatinin, .51 per cent as purine bases, and 11.4 per cent was insoluble in H Cl and NaOH.

It is significant that so large a portion of the excreted nitrogen consists of uric acid, which contains less hydrogen than any other known nitrogen compound excreted by animals. In so far as this has occurred, the insects have derived the highest possi-

ble amount of water from the oxidation of nutrients. It is also important that the amount of ammonia in the excrement has nearly the right relation to uric acid to form ammonium urate, a very insoluble compound which is voided solid with a minimum loss of water, and further, on account of the insolubility of ammonium urate, the toxic effects of both ammonia and uric acid are practically eliminated so that little water is wasted in their removal.

These insects are interesting, not only because they subsist entirely on air dried food containing little water, but especially because their food materials are wholly protein, consisting chiefly of keratin, a substance that is not attacked by the digestive enzymes of any other class of animals and is among the most resistant of animal products to decay. The analysis of the excrement shows that over 85 per cent of the excreted nitrogen is in a different form from that in the food material. Since these food materials are wholly protein in character, it is certain that the digestibility of hair or wool, by these insects, is at least 85 per cent of the amount consumed. The digestibility undoubtedly exceeds this because it is unlikely that all of the metabolized nitrogenous products were identified. It is noteworthy that the digestibility of keratin by these insects considerably exceeds the average digestibility of vegetable proteins by the higher animals.

Attempts have been made to extract from the moth larvae an enzyme capable of dissolving hair and wool but thus far without success. Only a few larvae were available for this purpose and further efforts will be made if a sufficient number of larvae can be obtained at one time.

#### THE BEE MOTH

(*Galleria mellonella* Linn)

The larvae of these insects subsist upon empty honey comb, which they perforate in all directions by tunnels and cover with a fine web which protects them from the attacks of bees. The mature larvae are about  $\frac{3}{4}$  inch long and  $\frac{1}{8}$  inch in diameter, and are very active. They are most abundant in abandoned hives and prefer the old dark colored comb to that which is clean and white. They will not eat clean melted wax.

A quantity of infested comb, sent to the Station by Carl H.

Hanson, of Elk Mound, Wis., supplied material for the following tests. The comb contained many larvae 1-4 to 3-4 inches long. The water content of the full grown larvae averaging 170 milligrams was found to be 57.30 per cent and that of medium sized larvae averaging 114 milligrams was 59.24 per cent, indicating the more active metabolism by the younger insects that were growing rapidly.

Some of the larvae were placed in a breeding cage, upon honey comb which they ate rapidly and in a short time went into the pupal state. The moths that issued from the cocoons deposited eggs on the comb and the sides of the cage which hatched in a few days. The larvae grew rapidly and in a few weeks passed into the pupal state. The honey comb, upon which the larvae fed, contained 1.85 per cent water, and 0.95 per cent nitrogen. The larvae had access to no water from any other source and must have derived practically all of the water required for their vital processes from metabolic changes in the food.

A piece of comb upon which eggs had been deposited was transferred from the cage to a desiccator, the air of which was dried by calcium chloride. They hatched as soon as those in the open air, large numbers of the larvae being seen crawling over the comb and the sides of the vessel after the fourth day. This condition continued until the nineteenth day, when no more live larvae could be seen. When the desiccator was opened on the twenty-third, twenty-eighth, and thirty-sixth days, a number of live larvae were found secreted in the comb. These larvae were neither so active nor so large as those grown in the open air, but they were larger than when hatched. The excrement found in the desiccator supplies additional evidence that these insects are able to assimilate dry food, in practically a dry atmosphere. Moreover they do this when first hatched, during a period of development when they are least able to withstand adverse conditions. It seems probable that these larvae, as well as those of the clothes moths, died at the first molting period.

Another piece of comb containing a number of larvae in different stages of development was placed in the desiccator. These larvae continued to grow; some of them spun cocoons, and 27 days after the comb was placed in the desiccator a moth appeared; another appeared on the ninety-third day and still

another on the one hundred twelfth day. During the next six weeks, six additional moths appeared in the desiccator and at this time live larvae were still present in the comb, and were growing. Since only four larvae were known to have been placed in the desiccator, at first, it is certain that some very small specimens escaped observation. It is possible that a few eggs introduced with the comb may have hatched and passed through every stage of development. It is certain that larvae which have passed the first molting period are capable of withstanding the increased evaporation induced by dry air, even at subsequent molting periods. At any rate they are far more resistant than when first hatched. In this case, practically all the water requirements must have been supplied through metabolic changes in the dry food, since the comb must have lost nearly all of its hygroscopic water in the desiccator.

Beeswax, the chief food of these insects is resistant to ordinary solvents. It is not noticeably hygroscopic, but it contains a high per cent of hydrogen and when assimilated and oxidized in respiration, it yields more than its weight of water. The nitrogen found in the comb, from which the insects derive all of their protein tissue, is probably due to adhering pollen grains. Nitrogen determinations, by Professor E. V. McCollum, in the honey comb used for these experiments and also in the full grown, live larvae, showed the nitrogen content of honey comb to be 0.95 per cent, and the nitrogen content of live larvae of the bee moth, 2.52 per cent.

Although the protein content of the food consumed by the larvae is low, the bodies of the insects contain fully as much nitrogen as is found in other classes of animals which feed upon a far narrower ration.

#### THE FOUR-SPOTTED PEA WEEVIL

(*Bruchus quadri-maculatus* Fabr.)

Specimens were found in a package of seed beans. These were transferred to a ventilated cage containing sound beans, and in about three months mature insects appeared, nearly every bean containing two or more perforations made by the larvae. It was impracticable to separate a sufficient number of small larvae from the beans for a reliable water determination. Three determinations in the adult weevils gave 48.92, 51.26, and

47.78 per cent. The water content of the larvae is undoubtedly several per cent higher than this. The beans upon which these fed exclusively contained 8.38 per cent water.

#### THE CONFUSED FLOUR BEETLE

(*Tribolium confusum* Duv.)

These were found in a package of ground red pepper. They were transferred to other vessels containing wheat bran and wheat flour, where they multiplied. In a few weeks insects in every stage of development were found in each of the vessels. The water content of the larvae was 62.43 per cent, and of the mature beetles 50.38 per cent. Water was not determined in the food materials, but it is not likely to have exceeded 10 per cent.

Mature insects were placed in a beaker containing wheat flour that had been dried in a steam oven, and the beaker put into a desiccator over sulphuric acid. The beetles burrowed continually in the dry flour and probably laid eggs although none were seen and no larvae were found in the flour. No dead beetles were seen until the twentieth day, the number increasing from this time, but live beetles were observed until the fifty-eighth day. It is quite certain that the beetles ate some of the dry flour, and that their life was prolonged thereby, since beetles confined in a vessel containing no food, in the open air where conditions were much more favorable, all starved to death in thirty-six days.

The natural life of the mature beetles is longer than most other mature insects as in shown by experiments made to determine whether they could subsist upon food materials containing only a single protein. The rations supplied to the beetles, in these experiments, consisted of one part of either zein or edestin and ten parts of starch, to which was added a little milk ash. A mixture of the two proteins was also used. In addition, the above rations were all duplicated with a little cinnamon oil added to each. The mixtures were placed in 100 c. c. flasks that were loosely stoppered, to prevent escape and to admit air. Ten mature beetles were placed in each flask. No dead beetles were seen in any of the flasks during the first six months, and they were not all dead at the end of 11½ months. No live beetles were seen after a year. There was apparently no differ-

ence in the effect of the different mixtures. No larvae were seen at any time in any of the flasks. It is not known whether eggs were deposited or not. This may have occurred and escaped observation, or possibly the eggs may have hatched and the young larvae been devoured by the beetles. In any case, the failure to reproduce is certain evidence that none of the rations were entirely suited to the needs of the insects. It is therefore probable that, under proper conditions, the normal life of the insects is more than a year.

#### MEDITERRANEAN FLOUR MOTH

(*Ephestia kuehniella* Zeller.)

Larvae were supplied by the department of economic entomology of the Station, to which they were sent for identification. The full grown larvae contained 64.22 per cent water. The water content of the food upon which the larvae fed was not determined, but it is well known that these insects pass through every stage of development upon food containing less than 10 per cent water.

A sample of wheat flour middlings, that originally contained 10.62 per cent. water, was infested with mites not identified. They could not be separated from adhering material. The sample had been kept several months, in a covered tin can, in the laboratory where the air is quite dry, and it is unlikely that it had absorbed water since the first determination was made. The water content of the mixture of mature insects, larvae and adhering material was 15.98 per cent; a portion of the flour, from which the insects were separated by sifting contained 9.88 per cent water. The increased water content of the flour is entirely due to the metabolic water produced by the respiration of the insects.

The water content of insect larvae that feed upon succulent food is naturally higher than in insects that eat only dry food.

In such cases the production of metabolic water is shown by a higher proportion of water in the larvae than in the green food they eat. This is well illustrated by water determinations in the larvae of the tobacco hornworm, (*Phlegethontius quinque-maculata*, Haworth.) The water content of full grown larvae was 85.65 per cent, of half grown larvae, 88.34 per cent and of the tobacco leaves which formed their food, 82.78 per cent.



Here as in all other cases, the younger larvae, in which the metabolic processes are most active, contain the higher per cent of water.

Similar determinations in larvae of the common green cabbage worm, (*Pieris rapae*, Linn.), showed the water content of full grown larvae to be 83.33 per cent, of medium sized larvae, 84.26 per cent, and of the cabbage leaves which formed their food 81.37 per cent. Here again the larger larvae contain the least water.

#### WATER REQUIREMENTS OF ANIMALS

The amount of free water required by animals depends, primarily, upon the species of animal, since this determines, within narrow limits, the nature of its food and the ultimate products of protein metabolism. Carbohydrates and fats, contained in food, may be completely oxidized through respiration, the products being carbon dioxide and water. The carbon dioxide is readily eliminated as a gas while the resulting water is added to the body fluids and utilized for the maintenance of the vital functions. The water produced in this way is more than sufficient to replace the normal loss of water through respiration and surface evaporation and since no poisonous substances are formed in the complete metabolism of carbohydrates or of fats, there would be no need of free water for maintenance, beyond the small amount always present in air-dry food, were it not for the accumulation of mineral constituents derived from the food and the poisonous nature of the end products of protein metabolism, the most of which must be removed in solution.

Where provision is made for the removal of these harmful waste products, in a solid form, as is the case with all insects that subsist upon air-dry materials, there is no need for a free water supply and there are good reasons for believing that animals of this class may develop and complete their life cycle while receiving no food except that which is perfectly dry.

On the other hand, when the end products of protein metabolism are soluble in the body fluids, the amount of water resulting from oxidation of organic nutrients is far too small to keep the solution of these substances in the body tissues below a toxic concentration. The danger is increased with animals

yielding milk and with birds laying eggs, since these products remove large amounts of water directly from the body fluids. Their production also demands the consumption of a considerable excess of protein above the amount required for normal maintenance, the waste products of which must also be removed. In these cases, an extra amount of water from external sources, proportional to the yields of milk or eggs must be supplied.

The energy expended in work may be wholly derived from the oxidation of carbohydrates and fats, as was first shown by experiments of Fick and Wislicenus in 1865 and later in experiments by Voit, Pettenkoffer and Park and still later in experiments of Argutinsky, Zuntz, and others. It is however, impracticable to supply, in food, sufficient carbohydrates and fats for the maintenance of an animal performing heavy work, without giving some extra protein, since the ordinary grains and feeds contain considerable protein. This surplus of protein is eliminated from the body as urea and an increase in the supply of water must be provided for the purpose.

The increased oxidation of nutrients during work results in the production of an increased amount of heat which, unless removed will raise the temperature of the body above normal. Danger from this source is obviated by an increased evaporation of water from the lungs and from the whole surface of animals that perspire freely. The water derived from the oxidation of nutrients is insufficient to replace the extra evaporation during work and more water from an external source must be supplied for this purpose than when an animal is at rest.

The water requirement of mature animals that excrete urea, when at rest, depends chiefly upon the amount of digestible protein consumed. For a strictly maintenance ration only sufficient protein need be assimilated to replace the loss of nitrogen from the tissues, and to furnish material for the natural loss of hair etc., if enough carbohydrates and fats are provided to meet the energy and heat requirements. Under these conditions, in which the urea excreted is a minimum and the metabolic water liberated in the tissues is a maximum, the supply of water from an external source may be small.

Serpents and other reptiles that live in arid regions and rarely if ever have access to water, except that contained in

their food are said by Vauquelin to excrete all of the waste nitrogen as salts of uric acid. The same is true of birds that live on desert islands where only salt water is available. It is essential that animals of these types should produce as much metabolic water as possible from the assimilated food, and the waste of water through the excretions should be reduced to a minimum. Since the food is largely protein both of these ends are attained by the excretion of uric acid which, as already stated, contains the least hydrogen of any nitrogenous substance excreted by animals so that the maximum amount of metabolic water has been derived from the food consumed. Uric acid and its salts, however, are practically insoluble in water and may be voided in a solid condition thereby conserving large quantities of water for other purposes that would be lost if the excreted product were soluble in water as is the case with urea, the usual form in which metabolized nitrogen is excreted.

Most large domestic animals excrete the metabolized nitrogen of their food as urea which is soluble in the body fluids and hence requires more water for its elimination than animals that excrete uric acid and its salts, which are insoluble and usually voided solid. It would be expected also that animals which excrete urea would require more water when the proportion of protein in the ration is increased. Upon this point Professor Henry<sup>18</sup> says, "Possibly due to their laxative nature, feeds rich in crude protein (bran, linseed meal, peas, etc.) cause a greater demand for water than starchy feeds." And again<sup>19</sup> "On protein-rich feeds the pig needs more water than when on starchy feeds." An experiment with wide and narrow rations by Armsby<sup>20</sup> shows that the cows fed on the narrow ration drank more water (this being supplied *ad libitum*) than those on a wider ration. There were two sets of experiments with different animals each extending over three weeks, two animals being fed in each case. Table XIX shows the average daily amounts of water consumed per animal, as well as the amount of water taken per pound of dry matter in the ration.

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<sup>18</sup> Feeds and Feeding, p. 63.

<sup>19</sup> Feeds and Feeding, p. 563.

<sup>20</sup> Wis. Expt. Sta. Rept. 1887, p. 136.

Table XX, compiled from results obtained by Georgeson of the Kansas Station in experiments with fattening steers, is copied from "Feeds and Feeding" p. 327.

TABLE XIX. INFLUENCE OF NUTRITIVE RATIO ON WATER INTAKE

Experiment	Period	Nutritive ratio	WATER CONSUMED	
			Total lbs. per day	Pounds water per lb. of dry matter
I.....	1	1:7.9	71.1	3.28
	2	1:11	61.1	3.09
	3	1:7.9	75.4	3.50
II.....	1	1:6.1	70.4	3.24
	2	1:4.9	74.7	3.49
	3	1:5.9	74.3	3.40

Many other experiments might be cited in support of the hypothesis that animals consume more water when the protein in the ration is increased. I believe it is a general principle, and that the function of the extra water is to facilitate the removal of urea from the system.

TABLE XX. WATER DRUNK IN WINTER BY FATTENING STEERS

Lot	Feed given	Water drunk	
		Lbs. daily per steer	Lbs. per lb. of feed
I.....	Corn meal, bran, shorts, oil meal with hay.....	79	2.5
II.....	Corn meal, molasses, and corn fodder.....	73	2.4
III...	Oil cake, hay.....	91	3.4
IV...	Ear corn, corn fodder.....	56	1.8

Humans and other large animals that excrete the waste nitrogen products of protein metabolism chiefly in the form of urea live only a short time if deprived of water, even when organic nutrients are supplied in abundance. They live much longer and suffer less when organic nutrients are withheld and water is supplied. This indicates that death, in the first instance, is not due to starvation since the necessary energy for maintaining vital functions is provided; the metabolic water is also more than sufficient to replace the water lost by evaporation and by respiration. The fact that an abundant supply of water pro-

longs life, although it contributes no energy to the system, indicates that death ensues when water is withheld, because of an accumulation of end products of metabolism, in this case urea etc, which cannot be eliminated by the small surplus of metabolic water available for this purpose. In other words, death results from uremic poisoning rather than from starvation.

Further evidence in this same line is supplied by reptiles, some species of insects, and birds that void the nitrogen end products as salts of uric acid, in a solid form. Many of these are capable of going without food or water for long periods and are apparently none the worse for the fast. In this case the metabolic water, together with that contained in the food is sufficient for all purposes, since water required for removing waste products is reduced to a minimum. I once knew of a hen that lived for six weeks without a particle of food or a drop of water and although extremely emaciated and weak when discovered, she quickly regained her strength and weight and was soon apparently as well as ever.

Hibernating animals, which subsist chiefly upon stored body fat and in which protein metabolism is reduced to a minimum, go for several months without food or water, metabolic water being ample for all their needs. It should be borne in mind in this connection that the oxidation of fats during destructive metabolism results in the production of a greater weight of water than the original weight of fats oxidized; also that respiration being at an extremely low ebb in this state, the loss of water through the lungs is very small. The low temperature tends to reduce evaporation from the skin to a minimum. Under these conditions the amount of metabolic water may be ample for removing injurious waste products. Another possibility is that animals in this state may be less sensitive to uremic poisoning than when active.

There are many animals that are able to go long periods without having access to water except that contained in their food, in which water usually amounts to less than 20 per cent of total weight, and the metabolic water derived from oxidation of organic nutrients. A notable example of this is the prairie dog which thrives in semi-arid regions. These small animals feed upon the native herbage which for months at a time is as dry as hay. It has been surmised that the burrows in which

they live extend to underground water courses, but this does not seem likely since in many of these regions wells must be sunk hundreds of feet before water is reached. It is more probable that they depend chiefly upon metabolic water. They feed mostly at night when the temperature is low and during the hottest hours of the day remain in their burrows where the air is more nearly saturated with moisture and evaporation is relatively small.

Mice will live for months upon air dried grain containing about 10 per cent of moisture, and under these conditions will give birth to young.

Sheep, when in pasture, or when a part of their ration consists of succulent feed, will live and grow for an indefinite period, without access to water, although they thrive better when water is available. In this case the thick covering of wool serves to diminish evaporation from the skin, and the relatively dry feces also tend to conserve water for other purposes.

The camel is able to carry heavy burdens over stretches of desert for many days without drinking. Before starting on a journey where water is not available, the drivers are particular to have the animals in good condition with the humps well stored with fat. At the end of a trip the animals are poor and the fat has mostly disappeared from the humps. The rations fed on these trips are chiefly carbohydrate concentrates consisting of dried fruits, bread, etc, containing little protein so that the nitrogen waste products from the food are small while the metabolic water is large. Evaporation of water from the skin is greatly reduced by a thick coat of fine hair and very little water is discharged with the feces, which are quite dry. It has been supposed that sufficient water was stored in the camel's stomach before starting to supply all demands of the trip. It seems likely, however that metabolic water derived from oxidation of body fats, and of food rich in carbohydrates is of far greater importance to the animal than the stored water.

An application of these principles would undoubtedly serve to prolong life, when suitable water for drinking is not available. In such cases, the food should consist of carbohydrates and fats. Proteins should not be used. In this way vital energy may be maintained with a minimum production of urea and a maximum amount of metabolic water, thereby greatly re-

ducing the danger from uremic poisoning. There need be no fear of starvation nor of permanent disability even when protein is entirely absent from a ration for several weeks if enough carbohydrate nutrients are provided to supply the necessary energy for maintaining the normal vital activities, and sufficient water is allowed for the removal of the small amount of urea arising under these conditions from the waste of body tissues. The water required for preventing uremic poisoning under these conditions is small and if the relative humidity of the surrounding air is high enough to prevent rapid evaporation of water from the body, the metabolic water arising from the oxidation of nutrients may be ample for the purpose.

#### SUMMARY

No life is possible except in the presence of water, which is always the most abundant constituent of active cells.

A portion of the water found in living organisms is derived from external sources either as free water, or as a normal constituent of the organic nutrients consumed. Another portion is produced within the organism through metabolic changes in its food and tissues, as a result of respiration.

The chief functions of water are to dissolve and transfer nutrients in solution to active cells, to remove the waste products of metabolism from these cells, and, in the chlorophyll bearing cells of plants, to supply material for the synthesis of organic nutrients.

The reactions involved in the conversion of nutrients to a soluble form adapted to the needs of living cells are all *hydrolytic* in nature. That is, they consist in adding the elements of water to the molecular structure of the nutrient. These changes are effected by the action of specific enzymes, peculiar to each type of organism, which are produced by respiring cells. The reactions involved are not directly dependent upon vital processes and for the most part they may be brought about by chemical means without contact with living tissues.

The reactions associated with the *growth* of tissue are all *dehydrating* in character. They are effected by energy set free through respiration. With few exceptions these reactions have not yet been brought about in the absence of living protoplasm.

Respiration may be direct or intramolecular. It is always

manifested by an evolution of carbon dioxide, the production of water, and the liberation of energy as heat. Direct respiration can occur only in the presence of free oxygen. In the absence of free oxygen intramolecular respiration is effected and energy set free through a rearrangement of the atoms of the molecules comprising the nutrients or the tissues, which results in the production of substances of a lower order than those originally present in the active cells among which carbon dioxide and water predominate.

The products of direct respiration (carbon dioxide and water) are not directly toxic to the cell. But if carbon dioxide is permitted to accumulate until it excludes free oxygen from the cells, intramolecular respiration intervenes and in addition to carbon dioxide and water, other products are formed which interfere with the normal activity of protoplasm. Unless these substances are removed as fast as formed, the death of the cell results in a short time. But if these substances are continually removed and replaced by suitable nutrients, vital processes may be carried on indefinitely with but little direct respiration. Growth never occurs except when direct respiration is possible.

The immediate effect of respiration is to remove by oxidation and dehydration a portion of the nutrients dissolved in the cell fluids, replacing them in part by water, and thus to reduce the concentration of the solution of nutrients within the cell wall below that in the surrounding fluids. In consequence of this there is established an osmotic movement of organic nutrients towards an active cell and of water in the opposite direction which is maintained so long as suitable nutrients are supplied and respiration is continued. It is this partial replacement of nutrients by metabolic water that determines the direction of the movement and insures a constant supply of nutrients to all respiring cells.

In every seed there are a few active cells which continue to respire and to perform all vital functions, so long as the seed is viable, and as respiration invariably results in the production of water, there must always be a small amount of water in every live seed or spore. It is impracticable to remove all of the water from a seed, except by the application of heat. Seeds of corn, exposed to air dried by sulphuric acid, for a period of nearly four years, still contain considerable water. The con-



tinual loss of dry matter, and evolution of carbon dioxide are ample evidence of respiration throughout the whole period.

Intramolecular respiration is incapable of maintaining the vital processes in seeds, for a long period. Direct respiration is therefore essential to the continued life and to the germination of a seed. For this reason, seeds intended for planting should always be stored under conditions which permit a rather free circulation of air. They should never be stored for a long time in bulk, in tight bins. The water content of seed grains should be reduced soon after harvest, to not more than 10 per cent, since the rate of respiration is dependent upon their water content.

The specific enzymes required for the conversion of the stored nutrients of a seed into available forms are absent in the immature seed, and only appear in the mature seed after direct respiration is established; the rate of their formation is proportional to the rate of direct respiration.

The water content of the sprouts of germinating seeds is always much higher than that of the remainder of a seed; this is due to the production of metabolic water by the rapid respiration of the young cells in these tissues, and to the fact that no respiration occurs in that portion of the seed in which the nutrients are stored.

Carbohydrates of various degrees of hydration are found in all plants; the highest state of hydration is represented by dextrose and levulose, the lowest by cellulose and starch, and between these, many intermediate forms occur. The first stable carbohydrate to appear in plants is starch which is formed initially in leaves by photosynthesis. This insoluble starch is hydrolized in the leaves by a diastatic enzyme and changed into a soluble carbohydrate, usually dextrose, and carried by osmosis to active cells in all parts of the plant. Within these cells, a portion of the dextrose is completely oxidized to carbon dioxide and water and through the energy liberated in the reaction other portions are dehydrated in various degrees giving rise to cellulose, starch, cane sugar, etc. The resulting cellulose remains within the cell to form the cell wall, the starch is deposited as a reserve nutrient to be drawn upon when food from other sources fails, the soluble waste products are removed from the cell by osmosis and mostly carried back to the leaves by the

sap where they are once more converted into suitable nutrients and distributed again to the active cells. This cycle may be completed an indefinite number of times by the same carbon nucleus until it is finally deposited as permanent tissue or is completely oxidized to supply energy for maintaining vital functions. At every round water is transferred in organic combination from the leaves to every growing cell. Nearly all of the water in the growing cells of a plant is brought to them, in this manner, in organic combination, and liberated within the cell walls by the process of respiration. The prevailing movement of liquid water is away from rather than toward these centers.

The free oxygen required for respiration during the germination of seeds may be derived from a solution of hydrogen peroxide in which the seeds are immersed. This method of testing germination has an advantage over the usual practice of placing the seeds between wet cloths, since the growth of molds, mildews, etc. which interfere with germination, is prevented.

Dry starch combines directly with water in a manner analogous to substances which crystallize with water of crystallization. This molecular combination always precedes the hydrolysis of starch to dextrose by a diastatic ferment.

The heat generated in the preliminary combination of starch with water is practically two thirds of the theoretical amount set free in the change from starch to dextrose. No change in temperature occurs when a boiled starch paste is hydrolized by diastase to dextrose, since the heat absorbed in the solution of the anhydrous dextrose formed is equal to that set free when hydrated starch is hydrolized.

The final ripening changes in most fruits proceed fully as rapidly after removal from the tree as when left undisturbed. These changes are the result of direct respiration of living cells in the fruit which continue to function after the fruit is picked. The increase in succulence during ripening is partly due to the production of metabolic water through respiration and partly to the increased solubility of the products formed. It is not due to water derived from the parent plant. The water content is proportionately greater in ripe fruit than in green fruit, in spite of considerable loss of water through evaporation, even through the fruit be ripened off the tree.

Fruits do not ripen normally when oxygen is excluded. In this case the changes are similar to those that occur in the en-siling of succulent plant tissues of any kind. They are usually associated with the production of organic acids of various kinds.

Water absorbed from the soil through the roots by capillarity passes quite directly through the tracheids to the leaves, picking up on the way by solution, carbon dioxide and other waste products of cellular respiration from all of the living tissues of the plant. By the action of light, all of these waste products in the leaves are reorganized into suitable nutrients and again distributed from cell to cell by osmosis.

The continual addition of carbon dioxide from respiring cells to the sap as it passes through the tracheids towards the leaves and the varying solubility of carbon dioxide in the sap with change of temperature, are most important factors in developing sap pressure and maintaining its movement at all periods of growth.

Through photosynthesis practically all of the waste products of plant metabolism are converted into nutrient substances and used again for maintaining vital processes. In this way, the loss of organic nutrients is reduced to a minimum and the rate of plant growth greatly augmented.

Animal cells respire in a similar manner to vegetable cells and with the same general effect. The oxidation of nutrients and production of metabolic water within the cell wall constantly maintains the solution of nutrients in the cell fluids at a lower concentration than in the blood which distributes nutrients to the tissues. This difference in concentration insures a constant movement by osmosis of soluble nutrients through the cell wall to replace the material that has been destroyed by respiration or removed from solution for the growth of tissue.

The most important difference between vegetable and animal metabolism is the inability of animals to reconvert the waste products of respiration into suitable nutrients. Most of these products are toxic to animal cells and must be eliminated from the tissues as fast as formed. This is especially the case with the waste products of protein metabolism. Most animals excrete these products in solution through the kidneys chiefly as urea, but insects, birds, and reptiles excrete insoluble salts of uric acid in a solid form with a minimum loss of water. It is sig-

nificant in this connection that uric acid contains the least hydrogen of any nitrogenous compound excreted by animals, thus securing for the animals that eliminate their nitrogenous waste products in this form the maximum amount of metabolic water.

Animals that excrete urea require a liberal supply of water, aside from that normally contained in the food, to keep the urea content of the blood below a toxic concentration. The water requirements of these animals depend largely upon the amount of protein consumed. On a protein free diet the metabolic water together with the free water contained in the food is sufficient to maintain all vital processes, to remove poisonous excretions, and to replace water lost in respiration and in evaporation from the body for long periods, as is shown by hibernating animals that subsist chiefly upon body fat.

Many varieties of insects and other animals that excrete the waste products of protein metabolism as salts of uric acid in solid form require no free water at any time, except the small amount present in air dried food, the water content of which is usually less than 10 per cent. This is possible because the insoluble nature of uric acid renders it but slightly poisonous and permits of its excretion with a minimum loss of water. This is the case with the clothes moths, the grain weevils, the bee moth, and a large number of insects that live upon air dried food throughout every stage of their development. The larvae of these insects contain from five to ten times the amount of free water contained in their food. Some of these insects are capable of living long periods upon dry food in an atmosphere containing no moisture. No doubt they would live indefinitely upon dry food if this could be supplied without exposure to dry air which enormously increases the loss of water by evaporation.

Metabolic water derived from the oxidation of organic nutrients would probably be sufficient for all animal needs were it not for the elimination of poisonous substances resulting from protein degeneration.



# Relation of Soil Bacteria to Evaporation

CONRAD HOFFMANN<sup>1</sup>

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## INTRODUCTION

Numerous articles and parts of many textbooks have been written dealing entirely with the water supply of soils, but in all cases the discussions concerning the subject have been confined largely to physical, and less frequently to chemical considerations. Mention is made of capillarity, gravity, viscosity, surface tension, evaporation, hygroscopic moisture, etc. The laws governing these factors have been very largely determined and are now facts known to many. But aside from the consideration of the physical and chemical agencies affecting soil moisture, there are certain biological factors in the soil which undoubtedly are closely connected with the movements of soil water. Owing to the prevailing tendency of the earlier soil bacteriologists to confine their efforts largely to problems concerned with the nitrogen supply of soils, and those more or less closely allied thereto, other fields of profitable investigation have been somewhat neglected. Thus, practically no work has been performed to investigate what relations, if any, exist between the movement of soil water and bacterial activities in the soil.

Capillarity is to a large extent dependent upon the surface tension of the soil water; thus the upward movement of water taking place as a result of capillarity in soils must also be influenced by the surface tension. It is accordingly reasonable to suppose that evaporation at the surface must therefore be influenced. It is a well known fact that the addition of certain fertilizers to soil increases the surface tension of the soil water. Soluble organic matter even in minute quantities, particularly

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<sup>1</sup> Much of the experimental work herein reported was performed under direction of the author, by J. E. Graul and A. A. Vass.

that of an oily nature, greatly reduces the surface tension. As bacteria in their metabolism undoubtedly form considerable quantities of soluble compounds both organic and inorganic, it is reasonable to suppose that the soil bacteria can and do, influence the rate of evaporation from soils, as well as the movement of soil water.

In recognition of this fact the work herein reported was undertaken to determine, if any exist, the true relation between the soil bacteria and the rate of evaporation of moisture from the soil.

Stigell<sup>2</sup> reports a few preliminary experiments relative to the influence of soil bacteria upon evaporation. His results indicate that the growth of bacteria exerts a retarding influence on the rate of evaporation. He attributes this retardation to the following possible factors: The absorption and subsequent retention of moisture by the bacteria themselves; and changes in the physical and chemical properties of the soil brought about by the metabolic activities of the bacteria.

Briefly, his method of procedure was to employ large Petri dishes containing a layer of quartz sand, moistened with bouillon cultures of various organisms. Weighings were made from day to day and the losses calculated as due to evaporation. Series of sterile dishes were similarly prepared to serve as controls. In this way he found that the presence of bacteria apparently retarded the rate of evaporation.

Similar experiments were next undertaken by the writer in Dr. Alfred Koch's laboratory at Göttingen. Here however, results were obtained which apparently contradicted those of Stigell. In fact, instead of a retardation there appeared to be an acceleration in the rate of evaporation.

#### DEVELOPMENT OF TECHNIQUE

It was found that the technique recommended by Stigell introduced a factor of error, which frequently was so large as to cover the slight differences which Stigell had obtained between sterile and inoculated plates. In order to secure more reliable results, it was found advisable to run at least four and preferably six plates to a set, instead of only one, using the average

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<sup>2</sup> *Centbl. Bakt. Abt. II*, 1908, **21**, p. 60.

for final results. Even where the utmost precautions to avoid errors were observed, wide variations were frequently obtained in the individual dishes of any given set.

Such factors as temperature and the humidity of the air could not be controlled under ordinary room conditions. These factors would of course, alter the rate of evaporation from day to day, being more rapid on a dry, sunshiny day than on a rainy, dark day. To avoid fluctuations due to air currents, proximity to radiators, etc., the experiments were conducted in a small room separated from the rest of the laboratory. All plates were placed under uniform conditions at equal distances from the walls of the room, and all plates in any given series were placed at the same level. The control and the normal plates were alternated on the shelves, rather than placing all normals or all controls adjacent to one another. In this way it was possible to expose all plates (normal and control) to identical conditions, and any fluctuations occurring would influence all dishes alike. Differences in the rate of evaporation between sets of the various series would then be attributed to differences in treatment rather than in exposure, and comparisons could thus be made with a greater degree of accuracy.

Several experiments were conducted, employing dishes ranging from 7 to 21 cm. in diameter, the soil layer varying from 2 to 10 cm. deep. While it was found that results were somewhat more uniform when a comparatively small evaporating surface and a deep soil layer were employed, the rate of evaporation under such conditions was so slow as to render their employment undesirable for the work in hand. The larger evaporating surfaces obviously increased the rate of loss due to evaporation, but at the same time increased the error, that is, the maximum variation between the individual dishes of any one set. By exercising care in the preparation of the plates this maximum variation between individual dishes was reduced to less than 2.5 grams where six plates were used in a set. It was finally decided to use dishes 19 to 22 cm. in diameter holding soil or sand 1.8 to 2.5 cm. deep. In any one experiment all dishes employed were of the same dimensions.

Part of the preliminary work was performed with quartz sand as a substratum, but far more uniform results were secured with ordinary dried and sieved soil as a substratum, this



being undoubtedly due to the fact that soil offers more continuous passages for capillary movements of the soil moisture. Where sand was employed, it was necessary to add nutrient solutions if luxuriant bacterial development was desired. This addition complicated the experiments, and produced conditions far from those normally and naturally existing in the field. Because of these facts soil was used instead of sand as a substratum, sand being used occasionally for comparisons. The soil used was allowed to air-dry, and was then passed through a twelve-mesh sieve. This was used throughout all experiments unless otherwise stated.

To measure the influence of bacterial growth upon the evaporation, it was necessary to run duplicate series kept under sterile conditions. As the plates were exposed directly to the air, they could not be protected from contamination after preparation. Owing to the nature of the experiment, it was impossible to cover the plates in any way to avoid this outside contamination. As the rate of evaporation from the soil is influenced by the extent of capillary action, and as capillarity is to a large extent dependent upon the surface tension of the soil water, it seemed inadvisable to add any disinfectants to the series to be kept sterile. The addition of any such soluble substances to the sterile series and not to the normal, where of course they could not be added if growth were desired, would, it was thought, alter the surface tension of the sterile series and so influence directly the capillarity, and indirectly the rate of evaporation. Ordinary heat sterilization would not suffice, for upon exposure to the air, contamination would immediately result.

Several experiments were conducted with a dilute mercuric chloride solution as a means of sterilizing the soils of one set in contrast to a duplicate set sterilized by heat. Twelve plates each containing 600 grams of soil were sterilized by heating. To each of six were then added 200 c. c. of a 1 to 1000 solution of mercuric chloride made with distilled water. To the other six, 200 c. c. of sterile distilled water were added. All plates were kept in a drying oven to avoid so far as possible the contamination of the set not treated with mercuric chloride. Thus all were identically treated except that mercuric chloride was added to one set of six. Daily weighings were made to determine

the losses of moisture due to evaporation. The averages of the six plates in each set are given in Table I.

It is apparent from the data that the mercuric chloride exerted no appreciable influence either in increasing or decreasing the rate of evaporation, as was at first expected. This being true, a dilute mercuric chloride solution was used to produce and maintain sterile conditions when desired.

TABLE I. INFLUENCE OF MERCURIC CHLORIDE SOLUTION ON RATE OF EVAPORATION

Sets	Loss of moisture in grams for the periods indicated							
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	Total
HgCl <sub>2</sub> set (Av. of 6 plates).....	12.2	6.5	8.8	7.9	7.2	12.5	14.3	69.4
H <sub>2</sub> O set (Av. of 6 plates).....	12.4	6.9	8.3	7.3	7.0	12.8	13.7	68.4
Difference in favor of HgCl <sub>2</sub> set.....	-0.2	-0.4	+0.5	+0.6	+0.2	-0.3	+0.6	+1.0

The technique finally adopted as a result of the preliminary work is briefly summarized as follows:

Circular dishes either 19.1 or 21 c. c. in diameter were employed. As a substratum, air-dried soil passed through a 12-mesh sieve was used in all cases unless otherwise stated. For each plate 500 or 600 grams of soil were used, which when uniformly packed and leveled, made a soil layer 2.8 cm. deep in the larger dishes and 3.2 cm. in the smaller dishes. To each of the plates were then added by means of a 50 c. c. pipette, 150 to 300 c. c. of distilled water depending upon the nature and amount of soil employed, the water being applied either pure or modified as indicated under the individual experiments. It was planned to have approximately 30% of moisture present initially in all experiments, thus affording ample moisture for vigorous bacterial multiplication. Great care was exercised to have the control sets differ from the normals in only the one respect desired, so that any differences occurring might be attributed to the one particular modification in treatment. In all cases the total initial weight was recorded and subsequent weighings were then made at more or less frequent intervals to determine the rate of evaporation. All weighings were made upon a torsion

balance weighing to within  $1/10$  of a gram. The results of the individual dishes of each set were then added and the average taken to indicate the loss due to evaporation occurring from period to period.

#### PROBLEM I. INFLUENCE OF GELATIN ON RATE OF EVAPORATION

Theoretically it would seem plausible to expect that bacterial growth, particularly if profuse, would tend to retard evaporation, as with profuse growth a more or less viscid condition obtains. The effect of such a condition would be purely physical and analogous to what one would expect from the addition of a dilute gelatin solution instead of distilled water. The moisture, it would seem, would be retained by the gelatinous nature of the bacterial growth in the first case and of gelatin itself in the latter.

Whether gelatin would occasion such a retention of moisture thereby preventing to a greater or less degree its evaporation, was thought worthy of investigation before proceeding further. Several experiments were accordingly performed employing a gelatin solution (approximately 2%) in contrast to ordinary distilled water, both under sterile conditions and in normal soil in which bacteria were allowed to develop. The results of these experiments are recorded in Tables II and III and represent the averages of the four plates used for each set.

It would appear that the gelatin solution employed had but little effect upon the evaporation. If any influence was exerted, it was at most a slight retardation, this being more evident in the normal soil sets. The differences however, between the normal distilled water and the dilute gelatin sets fall largely within the limits of the factor of error in all experiments, with the possible exception of Experiment 2 in Table III.

One can attribute but a slight retardation in the rate of evaporation to the presence of the gelatin, whereas the presence of bacteria, as subsequent experiments will reveal, causes an acceleration. The data in Tables II and III are rearranged in Tables IV and V to give a comparison between the sterile sets and the sets in which the normal soil bacteria were present and permitted to develop. One finds here a uniformity in all cases, namely that the normal sets all show an increased rate of evaporation in contrast to the sterile sets, whether gelatin was present

TABLE II. INFLUENCE OF GELATIN UPON EVAPORATION FROM STERILE SOIL

Sterile conditions were maintained throughout.

Interval	Loss of moisture in grams		Difference in favor of gelatin set	Total period	Total loss of moisture in grams		Total difference in favor of gelatin set
	With- out gel- atin	With gelatin			With- out gel- atin	With gelatin	
Experiment 1							
From start to 2nd day....	5.56	5.60	+0.04	2 days	5.56	5.60	+0.04
2nd to 4th day.....	4.54	4.60	+0.06	4 days	10.10	10.20	+0.1
4th to 7th day.....	7.80	7.80	0.00	7 days	17.90	18.00	+0.1
7th to 9th day.....	14.00	13.30	-0.7	9 days	31.90	31.30	-0.6
9th to 13th day.....	20.10	19.70	-0.4	13 days	52.00	51.00	-1.0
Experiment 2							
From start to 14th hr.....	10.67	10.00	-0.67	14 hrs.	10.67	10.00	-0.67
14th hr. to 24th hr.....	19.83	18.95	-0.88	1 day	30.50	28.95	-1.55
1st to 3rd day.....	15.00	16.35	+1.35	3 days	45.50	45.30	-0.20
3rd to 4th day.....	16.00	15.47	-0.53	4 days	61.50	60.77	-0.73
4th to 5th day.....	14.00	14.60	+0.60	5 days	75.50	75.37	-0.13
5th to 7th day.....	18.50	18.83	+0.33	7 days	94.00	94.20	+0.2
7th to 9th day.....	33.10	33.20	+0.10	9 days	127.10	127.40	+0.3
9th to 12th day.....	39.37	38.20	-1.17	12 days	166.47	165.60	-0.87
12th to 14th day.....	16.23	16.40	+0.17	14 days	182.70	182.00	-0.70

TABLE III. INFLUENCE OF GELATIN ON EVAPORATION FROM NORMAL SOIL

Soil under normal conditions with bacteria present.

Interval	Loss of moisture in grams		Differ- ence in favor of gelatin set	Total period	Total loss of moisture in grams		Total differ- ence in favor of gelatin set
	With- out gelatin	With gelatin			With- out gelatin	With gelatin	
Experiment 1							
From start to 2nd day....	6.50	5.90	-0.60	2 days	6.50	5.90	-0.60
2nd to 4th day.....	4.60	5.20	+0.60	4 days	11.10	11.10	0.00
4th to 7th day.....	8.00	8.30	+0.30	7 days	19.10	19.40	+0.30
7th to 9th day.....	13.50	11.20	-2.30	9 days	32.60	30.60	-2.00
9th to 13th day.....	21.00	19.70	-1.30	13 days	53.60	50.30	-3.30
Experiment 2							
From start to 14th hr....	11.00	11.95	+0.95	14 hrs..	11.00	11.95	+0.95
14th to 24th hr.....	21.56	20.55	-1.01	24 hrs..	32.56	32.50	-0.06
1st to 3rd day.....	17.71	17.31	-0.40	3 days	50.27	49.81	-0.46
3rd to 4th day.....	18.73	18.19	-0.54	4 days	69.00	68.00	-1.00
4th to 5th day.....	18.50	15.50	-3.00	5 days	87.50	83.50	-4.00
5th to 7th day.....	24.20	19.90	-4.30	7 days	111.70	103.40	-8.30
7th to 9th day.....	34.90	34.00	-0.90	9 days	146.60	137.40	-9.20
9th to 12th day.....	30.80	34.10	+3.30	12 days	177.40	171.50	-5.90
12th to 14th day.....	10.27	14.30	+4.03	14 days	187.67	185.80	-1.87

TABLE IV. INFLUENCE OF BACTERIAL DEVELOPMENT UPON EVAPORATION IN THE ABSENCE OF GELATIN

Rearrangement of data given in Tables II and III.

Interval	Loss of moisture in grams		Difference in favor of normal set	Total period	Total loss of moisture in grams		Total difference in favor of normal set
	Sterile	Normal			Sterile	Normal	
Experiment 1.							
From start to 2nd day...	5.56	6.50	+0.94	2 days	5.56	6.50	+0.94
2nd to 4th day.....	4.54	4.80	+0.06	4 days	10.10	11.10	+1.00
4th to 7th day.....	7.80	8.00	+0.20	7 days	17.90	19.10	+1.20
7th to 9th day.....	14.00	13.50	-0.50	9 days	31.90	32.60	+0.70
9th to 13th day.....	20.10	21.00	+0.90	13 days	52.00	53.60	+1.60
Experiment 2.							
From start to 14th hr....	10.67	11.00	+0.33	14 hrs.	10.67	11.00	+0.33
14th to 24th hr.....	19.83	21.56	+1.73	24 hrs.	30.50	32.56	+2.06
1st to 3rd day.....	15.00	17.71	+2.71	3 days	45.50	50.27	+4.77
3rd to 4th day.....	16.00	18.73	+2.73	4 days	61.50	69.00	+7.50
4th to 5th day.....	14.00	18.50	+4.50	5 days	75.50	87.50	+12.00
5th to 7th day.....	18.50	24.20	+5.70	7 days	94.00	111.70	+17.70
7th to 9th day.....	33.10	34.90	+1.80	9 days	127.10	146.60	+19.50
9th to 12th day.....	39.37	30.80	-8.57	12 days	166.47	177.40	+10.93
12th to 14th day.....	16.23	10.27	-5.96	14 days	182.70	187.67	+4.97

TABLE V. INFLUENCE OF BACTERIAL DEVELOPMENT UPON EVAPORATION IN THE PRESENCE OF GELATIN

Rearrangement of data given in Tables II and III.

Interval	Loss of moisture in grams		Difference in favor of normal set	Total period	Total loss of moisture in grams		Total difference in favor of normal set
	Sterile	Normal			Sterile	Normal	
Experiment 1							
From start to 2nd day....	5.60	5.90	+0.30	2 days	5.60	5.90	+0.30
2nd to 4th day.....	4.60	5.20	+0.60	4 days	10.20	11.10	+0.90
4th to 7th day.....	7.80	8.30	+0.50	7 days	18.00	19.40	+1.40
7th to 9th day.....	13.20	11.20	-2.10	9 days	31.30	30.60	-0.70
9th to 13th day.....	19.70	19.70	0.00	13 days	51.00	50.30	-0.70
Experiment 2							
From start to 14th hr....	10.00	11.95	+1.95	14 hrs.	10.00	11.95	+1.95
14th to 24th hr.....	18.95	20.55	+1.60	24 hrs.	28.95	32.50	+3.55
1st to 3rd day.....	16.35	17.31	+0.96	3 days	45.30	49.81	+4.51
3rd to 4th day.....	15.47	18.19	+2.72	4 days	60.77	68.00	+7.23
4th to 5th day.....	14.60	15.50	+0.90	5 days	75.37	83.50	+8.13
5th to 7th day.....	18.83	19.90	+1.07	7 days	94.20	103.40	+9.20
7th to 9th day.....	33.20	34.00	+0.80	9 days	127.40	137.40	+10.00
9th to 12th day.....	38.20	34.10	-4.10	12 days	165.60	171.50	+5.90
12th to 14th day.....	16.40	14.30	-2.10	14 days	182.00	185.80	+3.80

or not. It is further interesting to note that the differences in the rate of evaporation between the sterile and the normal sets were reduced, apparently by the presence of gelatin; a tendency as it were, of neutralizing the increased rate. This is in harmony with the results shown in Tables II and III where it ap-

pears that gelatin, probably due to its viscid nature, exerted a slight retardation upon the rate of evaporation.

## PROBLEM II. INFLUENCE OF SOIL BACTERIA ON RATE OF EVAPORATION

The influence of the bacterial flora of the soil upon evaporation was investigated before proceeding to any special modification of the work. Several soils were thus examined. They were air-dried and then passed through a twelve-mesh sieve and thoroughly mixed. In this way, a uniform and thoroughly representative sample was secured for each plate prepared. Eight plates for each soil were prepared, using 500-gram portions. To each of four of these, 200 c. c. distilled water were added to serve as a normal or inoculated set. To each of the other four, 200 c. c. of a 1:1000 mercuric chloride solution<sup>3</sup> were added. Thus the treatment of all plates was identical except that mercuric chloride was added to the sterile sets. In the normal or inoculated sets, bacterial development occurred; in the sterile sets, it was inhibited. As has been shown by preliminary work, the addition of the mercuric chloride solution, in the amounts employed, has no appreciable or measureable effect upon the loss of moisture. Accordingly, any differences which would occur between the inoculated and the sterile sets above mentioned must of necessity be attributed to the bacterial activities taking place in the inoculated plates.

The results of this series of experiments are recorded in condensed form in Tables VI to XI, the figures reported representing the average of the four plates in each set. Data are here given showing the loss of moisture for the various daily intervals as well as for the different total periods. Comparisons are also made between the sterile and the normal sets, the difference between the two for all soils being indicated. In those cases where the normal or inoculated sets showed a greater evaporation than the steriles, the differences are indicated by a plus sign; where the sterile exceeded the normal or inoculated, a minus sign is used to indicate the difference.

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<sup>3</sup> Made by adding 20 c. c. of a 1:100 solution to 180 c. c. of distilled water.

It is at once apparent that no constancy prevailed in the rate of evaporation from the various soils. They revealed considerable variation, probably due to differences in their physical and chemical composition.

TABLE VI. INFLUENCE OF NORMAL SOIL BACTERIA UPON RATE OF EVAPORATION FROM GREENHOUSE SOIL

Interval	Loss of moisture in grams		Difference in favor of inoculated set	Total period	Total loss of moisture in grams		Total difference in favor of inoculated set
	Sterile	Inoculated			Sterile	Inoculated	
1st 14 hrs.....	10.67	11.00	+0.33	14 hrs.	10.67	11.00	+0.33
14th to 24th hr.....	19.86	21.56	+1.70	24 hrs.	30.53	32.56	+2.03
1st to 3rd day.....	14.97	17.70	+2.73	3 days	45.50	50.26	+4.76
4th day.....	16.00	18.70	+2.70	4 days	61.50	68.96	+7.46
5th day.....	14.00	18.50	+4.50	5 days	75.50	87.46	+11.96
5th to 7th day.....	18.82	24.20	+5.38	7 days	94.32	111.66	+17.34
7th to 9th day.....	33.17	34.85	+1.68	9 days	127.49	146.51	+19.02
9th to 12th day.....	39.37	30.80	-8.57	12 days	166.86	177.31	+10.45
12th to 14th day.....	16.30	10.20	-6.10	14 days	183.16	187.51	+4.35

TABLE VII. INFLUENCE OF NORMAL SOIL BACTERIA UPON RATE OF EVAPORATION FROM WHITE QUARTZ SAND

Interval	Loss of moisture in grams		Difference in favor of inoculated set	Total period	Total loss of moisture in grams		Total difference in favor of inoculated set
	Sterile	Inoculated			Sterile	Inoculated	
1st 14 hrs.....	9.50	10.50	+1.00	14 hrs.	9.50	10.50	+1.00
14th to 24th hr.....	18.75	22.27	+3.52	24 hrs.	23.25	32.77	+9.52
1st to 3rd day.....	15.60	17.50	+1.90	3 days	43.85	50.27	+6.42
4th day.....	15.57	19.45	+3.88	4 days	59.42	69.72	+10.30
5th day.....	15.67	17.30	+1.63	5 days	75.09	87.02	+11.93
5th to 7th day.....	22.00	25.80	+3.80	7 days	97.09	112.82	+15.73
7th to 9th day.....	19.00	19.77	+0.77	9 days	116.09	132.59	+16.50

TABLE VIII. INFLUENCE OF NORMAL SOIL BACTERIA UPON RATE OF EVAPORATION FROM CLAY LOAM SOIL

Day interval	Loss of moisture in grams		Difference in favor of inoculated set	Total number of days	Total loss of moisture in grams		Total difference in favor of inoculated set
	Sterile	Inoculated			Sterile	Inoculated	
1st.....	28.72	26.10	-2.62	1	28.72	26.10	-2.62
2nd.....	13.45	14.90	+1.45	2	42.17	41.00	-1.17
3rd.....	18.35	20.10	+1.65	3	60.52	61.00	+0.48
4th.....	15.50	18.90	+3.40	4	76.02	79.90	+3.88
5th.....	15.70	16.70	+1.00	5	91.72	96.60	+4.88
6th.....	14.40	15.77	+1.37	6	106.12	112.37	+6.25
7th.....	12.55	15.15	+2.60	7	118.67	127.52	+8.85
8th.....	12.42	13.40	+0.98	8	131.09	140.92	+9.83

TABLE IX. INFLUENCE OF NORMAL SOIL BACTERIA UPON RATE OF EVAPORATION FROM SANDY SOIL

Day interval	Loss of moisture in grams		Difference in favor of inoculated set	Total number of days	Total loss of moisture in grams		Total difference in favor of inoculated set
	Sterile	Inoculated			Sterile	Inoculated	
1st.....	29.70	30.07	+0.37	1	29.70	30.07	+0.37
2nd.....	14.65	15.72	+1.07	2	44.35	45.79	+1.44
3rd.....	18.25	20.00	+1.75	3	62.60	65.79	+3.19
4th.....	15.47	17.10	+1.63	4	78.07	82.89	+4.82
5th.....	18.55	18.42	-0.07	5	96.62	101.31	+4.69
6th.....	17.11	17.90	+0.80	6	113.72	119.21	+5.49
7th.....	15.60	16.60	+1.00	7	129.32	135.81	+6.49
8th.....	14.70	15.42	+0.72	8	144.02	151.23	+7.21

TABLE X. INFLUENCE OF NORMAL SOIL BACTERIA UPON RATE OF EVAPORATION FROM FIELD CLAY SOIL.

Day interval	Loss of moisture in grams		Difference in favor of inoculated set	Total number of days	Total loss of moisture in grams		Total difference in favor of inoculated set
	Sterile	Inoculated			Sterile	Inoculated	
1st.....	18.0	15.2	-2.8	1	18.0	15.2	-2.8
2nd.....	18.0	15.7	-2.3	2	36.0	30.9	-5.1
3rd.....	21.2	20.7	-0.5	3	57.2	51.6	-5.6
4th.....	23.0	25.5	+2.5	4	80.2	77.1	-3.1
5th.....	23.0	25.0	+2.0	5	103.2	102.1	-1.1
6th.....	20.1	24.2	+4.1	6	123.3	126.3	+3.0
7th.....	19.6	23.4	+3.8	7	142.9	149.7	+6.8
8th.....	12.2	16.5	+4.3	8	155.1	166.2	+11.1
9th.....	14.6	14.8	+0.2	9	169.7	181.0	+11.3
10th.....	13.3	12.7	-0.6	10	183.0	193.7	+10.7
11th.....	5.8	4.2	-1.6	11	188.8	197.9	+9.1
12th.....	1.5	1.3	-0.2	12	190.3	199.2	+8.9
13th.....	1.2	1.2	0.0	13	191.5	200.4	+8.9

TABLE XI. INFLUENCE OF NORMAL SOIL BACTERIA UPON RATE OF EVAPORATION FROM MUCK SOIL

Day interval	Loss of moisture in grams		Difference in favor of inoculated set	Total number of days	Total loss of moisture in grams		Total difference in favor of inoculated set
	Sterile	Inoculated			Sterile	Inoculated	
1st to 2nd.....	33.40	33.80	+0.40	2	33.40	33.80	+0.40
2nd to 4th.....	40.10	44.50	+4.40	4	73.50	78.30	+4.80
5th day.....	19.40	20.00	+0.60	5	92.90	98.30	+5.40
5th to 7th.....	26.20	26.10	-0.10	7	119.10	124.40	+5.30
7th to 9th.....	21.49	21.30	-0.19	9	140.59	145.70	+5.11
9th to 12th.....	21.67	20.75	-0.92	12	162.16	166.45	+4.29
12th to 14th.....	9.60	8.45	-1.15	14	171.76	174.90	+3.14



One finds the maximum interval variation between sterile and normal sets in the greenhouse and the field clay soils, and the least variation with the sandy soil. On total variations the greenhouse soil exceeds all others showing as a maximum, 19.02 grams difference in favor of the inoculated set. It is interesting to note that in all cases, the rate of evaporation was more rapid in the inoculated set than in the sterile set.

This difference in favor of the inoculated plates is maintained with all the soils examined, and furthermore is so large as to exceed the factor of error.

A condition which is here evident, and which will become more so as the discussion of subsequent experiments progresses, is the fact that as the plates approach an air-dry condition the steriles show increased evaporation in contrast to the normal or inoculated plates; a more rapid rate of evaporation seems to occur in the steriles, and ultimately the total evaporation for both sets is again equal. This is due to the fact that the nearer air-dry the soil becomes, the more tenaciously the water is held. This condition is reached, as is apparent from the data in these and all other experiments, about the ninth day, from which time the total differences in favor of the inoculated set, begin to show a gradual decline. With the more rapid evaporation in the inoculated set during the earlier part of the experiment the air-dry condition is more rapidly approached, after which evaporation is much slower than in the sterile set which, due to the slower evaporation in the earlier stages, does not approach the air-dry condition so rapidly. Another feature worthy of mention is the fact that the greatest variations for the daily periods, between the sterile and inoculated sets usually occur between the fourth and sixth days of the experiments. It is well to bear this fact in mind, as subsequent experiments show that it is during this period that bacterial activity is greatest. The maximum interval variations in favor of the inoculated series coincide more or less closely with the period of greatest bacterial activity. As this condition holds for all soils experimented upon, one can justly conclude that the increased rate of evaporation in the inoculated series must be attributed to the bacteria themselves or to changes in the physical and chemical composition of the soil, occasioned by their activity. That this increase may be considerable has already been pointed out, amounting in the

case of the greenhouse soil to 19.02 grams, which is approximately 10% of the total moisture originally added.

The question naturally arises, "Do these conditions occur in the field?" Such wide differences would hardly be possible, but it must be admitted that bacteria can and undoubtedly do influence the rate of evaporation by affecting the movement of soil water. To what extent, cannot be said at this time. Whatever influence is exerted has probably been magnified by the nature of the experiments thus far conducted.

### PROBLEM III. RELATION BETWEEN BACTERIAL MULTIPLICATION AND INCREASED EVAPORATION

The fact that the maximum interval variation between the sterile and inoculated series usually occurred between the fourth and sixth days, rendered it advisable to determine experimentally whether any relation existed between the maximum loss of moisture and the highest bacterial development. With this end in view the following experiments were undertaken:

Two sets of six plates each were prepared, identical in every respect except that to one set 200 c. c. of a 1:1000 solution of mercuric chloride per plate had been added. Each plate of the other set received 200 c. c. of distilled water. All of the plates were then exposed. Upon three of the plates of each set, moisture and bacterial determinations were made daily, the germ content being then reduced to the dry basis. The other three plates of each set were left undisturbed, but were weighed daily to ascertain the rate of the loss of moisture. In this way data were secured giving the daily loss of moisture and the germ content of the soils on the respective days. It was thus possible to make a direct comparison between the loss of moisture due to evaporation, and the germ content of the soil. These experiments were repeated employing a dilute bouillon solution for moistening purposes in place of the ordinary distilled water which had been employed in all previous experiments. It was thought that the addition of this bouillon would enhance bacterial activity to a large extent and perhaps magnify the differences in the rate of evaporation. All data in connection with these two experiments are reported in Tables XII and XIII. These reveal the fact observed in the previous experiments, that

TABLE XII. RELATION BETWEEN BACTERIAL COUNT AND EVAPORATION  
Distilled water used to moisten soil. Ordinary soil infusion used for inoculation.

Day interval	Bacterial count in millions per gram	Loss of moisture in grams		Difference in favor of inoculated set	Total number of days	Loss of moisture in grams		Difference in favor of inoculated set
		Sterile	Inoculated			Sterile	Inoculated	
Soil infusion No. 1.								
1st.....	2.6	19.76	19.70	-0.06	1	19.76	19.70	-0.06
2nd.....		20.60	23.90	+3.30	2	40.36	43.60	+3.36
3rd.....	12.45	22.30	23.80	+1.50	3	62.66	67.40	+4.86
4th.....	20.90	21.30	25.60	+4.30	4	83.96	93.00	+9.16
5th.....	45.80	13.30	18.00	+4.70	5	97.26	111.00	+13.86
6th.....	35.45	13.90	20.20	+6.30	6	111.16	131.20	+20.16
7th.....	35.40	14.00	18.50	+4.50	7	125.16	149.70	+24.66
8th.....	30.20	12.50	15.00	+2.50	8	137.66	164.70	+27.16
Soil infusion No. 2.								
1st.....	5.3	18.0	15.2	-2.8	1	18.0	15.2	-2.8
2nd.....	9.7	18.0	15.7	-2.3	2	36.0	30.9	-5.1
3rd.....	18.7	21.2	20.7	-0.5	3	57.2	51.6	-5.6
4th.....	10.8	23.0	25.5	+2.5	4	80.2	77.1	-3.1
5th.....	9.0	23.0	25.0	+2.0	5	103.2	102.1	-1.1
6th.....	8.3	20.1	24.2	+4.1	6	123.3	126.3	+3.0
7th.....	6.3	19.6	23.4	+3.8	7	142.9	149.7	+6.8
8th.....	5.6	12.2	16.5	+4.3	8	155.1	166.2	+11.1
9th.....	4.5	14.6	14.8	+0.2	9	169.7	181.0	+11.3
10th.....	4.6	13.3	12.7	-0.6	10	183.0	193.7	+10.7
11th.....		5.8	4.2	-1.6	11	188.8	197.9	+9.1
12th.....		1.5	1.3	-0.2	12	190.3	199.2	+8.9
13th.....		1.2	1.2	0.0	13	191.5	200.4	+8.9

TABLE XIII. RELATION BETWEEN BACTERIAL COUNT AND EVAPORATION

Dilute bouillon used to moisten soil. Ordinary soil infusion used for inoculation.

Day interval	Bacterial count in millions per gram	Loss of moisture in grams		Difference in favor of inoculated set	Total number of days	Loss of moisture in grams		Difference in favor of inoculated set
		Sterile	Inoculated			Sterile	Inoculated	
1st.....	3.5	19.6	18.2	-1.4	1	19.6	18.2	-1.4
2nd.....	35.0	17.6	16.7	-0.9	2	37.2	34.9	-2.3
3rd.....	1500.0	17.4	19.1	+1.7	3	54.6	54.0	-0.6
4th.....	10000.0	17.0	15.2	-1.8	4	71.6	69.2	-2.4
5th.....	10000.0	19.8	20.0	+0.2	5	91.4	89.2	-2.2
6th.....	8000.0	16.8	18.0	+1.2	6	108.2	107.2	-0.2
7th.....	8000.0	17.6	20.2	+2.6	7	125.8	127.4	+1.6
8th.....	7500.0	13.9	14.9	+1.0	8	139.7	142.3	+2.6
9th.....	6000.0	15.5	18.1	+2.6	9	155.2	164.4	+9.2
10th.....	3400.0	14.0	16.0	+2.0	10	169.2	176.4	+7.2
11th.....	175.0	14.2	19.5	+5.3	11	183.4	195.9	+12.5
12th.....	20.0	14.2	17.7	+3.5	12	197.6	213.6	+16.0
13th.....	12.8	10.8	10.1	-0.7	13	208.4	223.7	+15.3
14th.....	7.0	7.9	7.6	-0.3	14	216.3	231.3	+15.0
15th.....		9.9	7.4	-2.5	15	226.2	238.7	+12.5

the maximum daily differences invariably occurred between the fourth and sixth days and that these maximum differences run more or less parallel to the maximum germ contents of the soils, or immediately follow them. As was to be expected in the case where dilute bouillon had been employed, a most marked bacterial multiplication occurred, amounting on the fourth and fifth days to over 10,000,000,000 per gram of soil, but in this case such parallelism between maximum daily variation and maximum germ content was not found. The extensive bacterial development probably fixed, so to speak, a large percentage of the moisture in the protoplasm of the bacterial cells, holding and preventing it from evaporating. In the other cases, where the bacterial multiplication was not so extreme, we find a close parallelism between maximum germ content and maximum interval variation, a fact which apparently indicates that the bacterial activities are responsible for the increased rate of evaporation in the normal or inoculated series.

#### PROBLEM IV. EFFECT OF REMOISTENING ON INCREASED EVAPORATION

In an endeavor to demonstrate still further the part played by the bacteria in causing the increased rate of evaporation, a slight modification of the previous experiments was performed by restoring to their original weights several series of plates after they had been exposed to evaporation. It was thought that thus the initial number of bacteria present would be greater, and that accordingly a more active evaporation would occur.

On comparing the data given in Table XIV one finds in every case a decided increase in the total rate of evaporation, over the increase shown in the initial experiment, even where modified by the different treatments as indicated.

A greater rate of evaporation in the inoculated series after remoistening than initially, is to be noted in all three experiments. These substantiate the evidence that bacteria are responsible directly or indirectly for the increased evaporation noted in all cases in the normal soils. While no germ content determinations were made upon the soils before and after remoistening, it is reasonable to suppose that the germ content was greater after remoistening than prior thereto. With this probable greater bacterial content, a greater difference in rate of evaporation was observed. It is further interesting to note that

the maximum daily difference in favor of the inoculated set falls between the fifth and sixth days, results which are in harmony with the previous experiments.

TABLE XIV. INCREASED EVAPORATION DUE TO REMOISTENING

Day interval	Interval increase of normal over sterile expressed in grams		Total number of days	Total increase of normal over sterile expressed in grams	
	Initially	After remoistening		Initially	After remoistening
1st.....	+2.1	-0.9	1	+2.1	-0.9
2nd to 4th.....	+3.8	+4.3	4	+5.9	+3.4
4th to 5th.....	+2.2	+2.9	5	+8.1	+6.3
5th to 7th.....	+0.1	+3.7	7	+8.2	+10.0
7th to 9th.....	-0.4	+4.7	9	+7.8	+14.7
9th to 12th.....	-3.2	.....	12	+4.6	.....
12th to 14th.....	-1.2	.....	14	+3.4	.....
1st.....	+1.0	+2.6	1	+1.0	+2.6
2nd to 4th.....	+7.0	+4.4	4	+8.0	+7.0
4th to 5th.....	+4.0	+3.0	5	+12.0	+10.0
5th to 7th.....	0.0	+6.4	7	+12.0	+16.4
7th to 9th.....	+0.6	+0.1	9	+12.6	+16.5
9th to 12th.....	-7.1	.....	12	+5.5	.....
12th to 14th.....	-1.5	.....	14	+4.0	.....
1st.....	-1.8	+0.8	1	-1.8	+0.8
2nd to 4th.....	+5.3	+1.3	4	+3.5	+2.1
4th to 5th.....	+3.3	+4.2	5	+6.8	+6.3
5th to 7th.....	-2.3	+4.3	7	+4.5	+10.6
7th to 9th.....	0.0	+1.5	9	+4.5	+12.1
9th to 12th.....	-4.0	.....	12	+0.5	.....
12th to 14th.....	+0.5	.....	14	+1.0	.....

## PROBLEM V. INFLUENCE OF ORGANIC MATTER ON EVAPORATION

It will be remembered that the work on the normal soil bacteria was performed with different types of soil as substrata from which evaporation was to take place, and that upon these the amount of increased evaporation due to the presence of bacteria varied with the type of soil employed. For example, the increased rate of evaporation from a sandy soil was not as great as that from a clay or greenhouse soil. These variations are probably due to differences in composition of the soils, both physical and chemical. That the presence or absence of organic matter is important from the physical standpoint in determining texture, water-holding capacity, and capillarity of soils is known. It would seem that its presence would also influence the results of these experiments.

What effect the addition of varying amounts of organic mat-

ter to a given soil would have, was not known, and was thought worthy of investigation. For this purpose the following experiments were conducted.

Two groups of three series each were prepared; one group was kept sterile in the usual manner, the other was inoculated with a soil infusion. The series in each group received 2%, 1%, and no blood meal respectively. After proper moistening all were exposed under uniform conditions and the losses occurring due to evaporation recorded.

TABLE XV. INFLUENCE OF BLOOD MEAL ON EVAPORATION FROM SOIL

Changes in rate of evaporation caused by the addition of varying amounts of blood meal to clay lean soil.

Day interval	Loss of moisture in grams		Difference in favor of inoculated set	Total num- ber of days	Loss of moisture in grams		Difference in favor of inoculated set
	Sterile	Inocu- lated			Sterile	Inocu- lated	
Series I							
<i>No blood meal</i>							
1st to 2nd.....	29.1	31.2	+2.1	2	29.1	31.2	+2.1
2nd to 4th.....	35.4	39.2	+3.8	4	64.5	70.4	+5.9
5th.....	21.2	23.4	+2.2	5	85.7	93.8	+8.1
5th to 7th.....	31.7	31.8	+0.1	7	117.4	128.6	+11.2
7th to 9th.....	35.6	35.2	-0.4	9	153.0	160.8	+7.8
9th to 12th.....	28.3	25.1	-3.2	12	181.3	185.9	+4.6
12th to 14th.....	8.3	7.1	-1.2	14	189.6	193.0	+3.4
Series II							
<i>1% blood meal</i>							
1st to 2nd.....	28.1	29.1	+1.0	2	28.1	29.1	+1.0
2nd to 4th.....	36.0	43.0	+7.0	4	64.1	72.1	+8.0
5th.....	20.3	24.3	+4.0	5	84.4	96.4	+12.0
5th to 7th.....	34.3	34.3	0.0	7	118.7	130.7	+12.0
7th to 9th.....	31.0	31.6	+0.6	9	149.7	162.3	+12.6
9th to 12th.....	28.4	21.3	-7.1	12	178.1	183.6	+5.5
12th to 14th.....	8.7	7.2	-1.5	14	186.8	190.8	+4.0
Series III							
<i>2% blood meal</i>							
1st to 2nd.....	31.5	29.7	-1.8	2	31.5	29.7	-1.8
2nd to 4th.....	37.3	42.6	+5.3	4	68.8	72.3	+3.5
5th.....	21.6	24.9	+3.3	5	90.4	97.2	+6.8
5th to 7th.....	33.8	31.5	-2.3	7	124.2	128.7	+4.5
7th to 9th.....	31.7	31.7	0.0	9	155.9	160.4	+4.5
9th to 12th.....	25.5	21.5	-4.0	12	181.4	181.9	+0.5
12th to 14th.....	8.1	8.6	+0.5	14	189.5	190.5	+1.0

From the data submitted in Tables XV and XVI it is seen that the maximum difference between the inoculated and sterile sets for any given period was obtained in the series containing 1% blood meal. This held true after remoistening the plates and again subjecting them to evaporation. In the presence of 2% blood meal the difference in favor of the inoculated series is not so marked. The data showing the total increased evaporation due to inoculation for the three series are given in Table XVII.

At the close of the experiments with blood meal all plates were restored to their original weight by the addition of water and then subjected to evaporation a second time. The results are indicated in Table XVI.

TABLE XVI. INFLUENCE OF BLOOD MEAL ON EVAPORATION AFTER REMOISTENING

Day interval	Loss of moisture in grams		Difference in favor of inoculated set	Total number of days	Loss of moisture in grams		Difference in favor of inoculated set
	Sterile	Inoculated			Sterile	Inoculated	
Series I <i>No blood meal</i>							
1st.....	13.7	12.8	-0.9	1	13.7	12.8	- 0.9
2nd.....	17.6	21.9	+4.3	2	31.3	34.7	+ 3.4
3rd.....	12.9	15.8	+2.9	3	44.2	50.5	+ 6.3
3rd to 5th.....	34.4	38.1	+3.7	5	78.6	88.6	+10.0
5th to 8th.....	38.6	43.3	+4.7	8	117.2	131.9	+14.7
Series II <i>1% blood meal</i>							
1st.....	13.7	16.3	+2.6	1	13.7	16.3	+ 2.6
2nd.....	17.8	22.2	+4.4	2	31.5	38.5	+ 7.0
3rd.....	13.7	16.7	+3.0	3	45.2	55.2	+10.0
3rd to 5th.....	32.7	39.1	+6.4	5	77.9	94.3	+16.4
5th to 8th.....	40.6	40.7	+0.1	8	118.5	135.0	+16.5
Series III <i>2% blood meal</i>							
1st.....	13.1	13.9	+0.8	1	13.1	13.9	+ 0.8
2nd.....	19.4	20.7	+1.3	2	32.5	34.6	+ 2.1
3rd.....	13.5	17.7	+4.2	3	46.0	52.3	+ 6.3
3rd to 5th.....	34.8	39.1	+4.3	5	80.8	91.4	+10.6
5th to 8th.....	40.0	41.5	+1.5	8	120.8	132.9	+12.1

The comparison of results before and after remoistening given in Table XVII, reveals close similarity. In both, Series II shows the maximum difference in rate of evaporation, whereas Series III containing 2% blood meal does not show a differ-

TABLE XVII. TOTAL INCREASE IN GRAMS IN EVAPORATION DUE TO INOCULATION IN THE THREE BLOOD MEAL SERIES

Number of days	Series I No blood meal	Series II 1% blood meal	Series III 2% blood meal
<b>Before remoistening</b>			
2.....	+ 2.1	+ 1.0	- 1.8
4.....	+ 5.9	+ 8.0	+ 3.5
5.....	+ 8.1	+12.0	+ 6.8
7.....	+ 8.2	+12.0	+ 4.5
9.....	+ 7.8	+12.8	+ 4.5
12.....	+ 4.6	+ 5.5	+ 0.5
14.....	+ 3.4	+ 4.5	+ 1.0
<b>After remoistening</b>			
1.....	- 0.9	+ 2.6	+ 0.8
2.....	+ 3.4	+ 7.0	+ 2.1
3.....	+ 6.3	+10.0	+ 6.3
5.....	+10.0	+16.4	+10.6
8.....	+14.7	+16.5	+12.1

ence so great as Series I which received no blood meal. Attention has already been called to the fact that the differences are greater after remoistening than before.

A repetition of the experiment with blood meal, but substituting barnyard manure (dried and ground up fine so as to pass through a twelve-mesh sieve) gave the results indicated in Table XVIII and summarized in Table XIX.

TABLE XVIII. EFFECT OF BARNYARD MANURE ON EVAPORATION  
Varying amounts of finely ground manure were added to clay loam soil, soil infusion serving as inoculating material.

Day interval	Loss of moisture in grams		Difference in favor of inoculated set	Total num- ber of days	Loss of moisture in grams		Difference in favor of inoculated set
	Sterile	Inocu- lated			Sterile	Inocu- lated	
Series I. No manure							
1st to 2nd .....	32.80	36.90	+4.10	2	32.80	36.90	+4.10
2nd to 4th .....	46.43	47.46	+1.03	4	79.23	84.36	+5.13
5th .....	21.96	25.80	+3.84	5	101.19	110.16	+8.97
5th to 7th .....	30.93	33.66	+2.73	7	132.12	143.82	+11.70
7th to 9th .....	34.26	33.28	-1.00	9	166.38	177.08	+10.70
9th to 12th .....	22.43	15.30	-7.13	12	188.81	192.38	+3.57
12th to 14th .....	2.56	1.43	-1.13	14	191.37	193.81	+2.44
Series II. 1% manure							
1st to 2nd .....	36.20	34.66	-1.54	2	36.20	34.66	-1.54
2nd to 4th .....	43.76	40.73	-3.03	4	79.96	75.39	-4.57
5th .....	25.66	23.76	-1.90	5	105.62	99.15	-6.47
5th to 7th .....	33.20	34.06	+0.86	7	138.82	133.21	-5.61
7th to 9th .....	32.06	31.66	+0.20	9	170.88	165.07	-5.81
9th to 12th .....	21.20	22.90	+1.70	12	192.08	187.97	-4.11
12th to 14th .....	2.56	3.90	+1.34	14	194.64	191.81	-2.83
Series III. 2% manure							
1st to 2nd .....	35.43	35.66	+0.23	2	35.43	35.66	+0.23
2nd to 4th .....	46.10	42.86	-3.24	4	81.53	78.52	-3.01
5th .....	22.80	25.40	+2.60	5	104.33	103.92	-0.41
5th to 7th .....	35.03	33.00	-2.03	7	139.36	136.92	-2.44
7th to 9th .....	32.16	32.90	+0.74	9	171.52	169.82	-1.70
9th to 12th .....	19.00	19.96	+0.96	12	190.52	189.78	-0.74
12th to 14th .....	2.96	3.10	+0.14	14	193.48	192.88	-0.60

TABLE XIX. INFLUENCE OF MANURE ON TOTAL EVAPORATION  
Difference between inoculated and sterile sets expressed in grams.

Number of days	Series I No manure	Series II 1% manure	Series III 2% manure
2.....	+4.10	-1.54	+0.23
4.....	+5.13	-4.57	-3.01
5.....	+8.97	-6.47	-0.41
7.....	+11.70	-5.61	-2.44
9.....	+10.70	-5.81	-1.70
12.....	+3.57	-4.11	-0.74
14.....	+2.44	-2.83	-0.60



TABLE XX. INFLUENCE OF ADDITION OF BLOOD MEAL

Day interval	Loss of moisture in grams		Difference in favor of inoculated set	Total number of days	Loss of moisture in grams		Difference in favor of inoculated set
	Sterile	Inoculated			Sterile	Inoculated	
Series I No blood meal							
1st.....	10.6	10.5	-0.1	1	10.6	10.5	-0.1
2nd.....	6.2	5.8	-0.4	2	16.8	16.3	-0.5
3rd & 4th.....	16.6	17.3	+0.7	4	33.4	33.6	+0.2
5th.....	8.8	8.6	-0.2	5	42.2	42.2	0.0
6th.....	8.5	9.6	+1.1	6	50.7	51.8	+1.1
7th.....	9.6	11.2	+1.6	7	60.3	63.0	+2.7
8th.....	6.6	6.8	+0.2	8	66.9	69.8	+2.9
9th.....	8.3	8.6	+0.3	9	75.2	78.4	+3.2
10th & 11th.....	16.8	17.8	+1.0	11	92.0	96.2	+4.2
12th & 13th.....	13.5	14.0	+0.5	13	105.5	110.2	+4.7
14th & 15th.....	13.0	15.2	+2.2	15	118.5	125.4	+6.9
16th, 17th & 18th.....	21.0	24.5	+3.5	18	139.5	149.9	+10.4
Series II + 1% blood meal							
1st.....	10.6	10.6	0.0	1	10.6	10.6	0.0
2nd.....	6.5	6.2	-0.3	2	17.1	16.8	-0.3
3rd & 4th.....	16.2	17.0	+0.8	4	33.3	35.8	+0.5
5th.....	8.6	9.0	+0.4	5	41.9	42.8	+0.9
6th.....	8.8	9.0	+0.2	6	50.7	51.8	+1.1
7th.....	9.6	10.6	+1.0	7	60.3	62.4	+2.1
8th.....	6.6	6.8	+0.2	8	66.9	69.2	+2.3
9th.....	8.0	8.6	+0.6	9	74.9	77.8	+2.9
10th & 11th.....	16.2	19.0	+2.8	11	91.1	96.8	+5.7
12th & 13th.....	13.0	14.3	+1.3	13	104.1	111.1	+7.0
14th & 15th.....	13.0	14.0	+1.0	15	117.1	125.1	+8.0
16th, 17th & 18th.....	22.6	24.2	+1.6	18	129.7	149.3	+9.6
Series III + 2% blood meal							
1st.....	12.2	11.5	-0.7	1	12.2	11.5	-0.7
2nd.....	6.2	6.5	+0.3	2	18.4	18.0	-0.4
3rd & 4th.....	15.5	16.3	+0.8	4	33.9	34.3	+0.4
5th.....	8.5	9.0	+0.5	5	42.4	43.3	+0.9
6th.....	10.0	9.6	-0.4	6	52.4	52.9	+0.5
7th.....	9.8	9.5	-0.3	7	62.2	62.4	+0.2
8th.....	7.3	7.6	+0.3	8	69.5	70.0	+0.5
9th.....	8.0	8.8	+0.8	9	77.5	78.8	+1.3
10th & 11th.....	17.8	21.2	+3.4	11	95.3	100.0	+4.7
12th & 13th.....	14.0	16.2	+2.2	13	109.8	113.2	+3.4
14th & 15th.....	13.2	15.8	+2.6	15	122.5	132.0	+9.5
16th, 17th & 18th.....	23.0	27.0	+4.0	18	145.5	159.0	+13.5
Series IV + 3.5% blood meal							
1st.....	11.5	11.8	+0.3	1	11.5	11.8	+0.3
2nd.....	5.8	6.5	+0.7	2	17.3	18.3	+1.0
3rd & 4th.....	15.6	16.6	+1.0	4	32.9	34.9	+2.0
5th.....	9.3	9.2	-0.1	5	42.2	44.1	+1.9
6th.....	8.6	9.2	+0.6	6	50.8	53.3	+2.5
7th.....	10.0	9.6	-0.4	7	60.8	62.9	+2.1
8th.....	7.2	8.0	+0.8	8	68.0	70.9	+2.9
9th.....	8.6	8.8	+0.2	9	76.6	79.7	+3.1
10th & 11th.....	17.2	21.5	+4.3	11	93.8	101.2	+7.4
12th & 13th.....	13.0	16.8	+3.8	13	106.8	118.0	+11.2
14th & 15th.....	13.0	13.5	+0.5	15	119.8	131.5	+11.7
16th, 17th & 18th.....	22.8	29.2	+6.4	18	142.6	160.7	+18.1
Series V + 5% blood meal							
1st.....	12.6	12.2	-0.4	1	12.6	12.2	-0.4
2nd.....	5.6	6.5	+0.9	2	18.2	18.7	+0.5
3rd & 4th.....	14.6	15.5	+0.9	4	32.8	34.2	+1.4
5th.....	9.2	9.5	+0.3	5	42.0	43.7	+1.7
6th.....	8.6	9.6	+1.0	6	50.6	53.3	+2.7
7th.....	9.5	9.6	+0.1	7	60.1	62.9	+2.8
8th.....	7.3	7.8	+0.5	8	67.4	70.7	+3.3
9th.....	8.5	9.3	+0.8	9	75.9	80.0	+4.1
10th & 11th.....	18.3	21.2	+2.9	11	94.2	108.7	+14.5
12th & 13th.....	14.5	18.2	+3.7	13	101.2	119.4	+18.2
14th & 15th.....	13.5	16.6	+3.1	15	114.7	136.0	+21.3
16th, 17th & 18th.....	24.5	27.5	+3.0	18	139.2	163.5	+24.3

The results here obtained seemingly contradict all previous work, for in the series receiving manurial additions, the sterile plates exceed the normal or inoculated ones in the rate of evaporation. This is true in both cases where manure was added. It is noteworthy, however, that the untreated series showed an acceleration in the rate of evaporation in the inoculated plates which harmonizes with the results of previous work. The cause of the reduction in the rate of evaporation may possibly be explained by the chemical composition of the manure and the by-products formed from it by bacterial activity. If these by-products are of such a consistency as to occasion a reduction in the surface tension of the soil water, then a movement of the moisture away from the surface of the soil plates would result. Such a movement would cause a reduction in the amount of water exposed to evaporation, and thus would retard the rate of evaporation.

The possibility of error in the results of experiments on the influence of the addition of organic substances, particularly that of manure, warranted a repetition of the same. These were performed on a somewhat larger scale, five series of plates being prepared. Both blood meal and manure were again employed using 1%, 2%, 3.5% and 5%, respectively, in contrast to the control series which received no additions. The results of these experiments are given in Tables XX and XXI.

In the case of blood meal (Table XX), note how closely the results in all series agree. Up to the ninth day there is but little difference; thereafter, however, the series receiving additions of blood meal show increased rates of evaporation due to inoculation, in contrast to the series receiving no blood meal. This difference is most marked with the larger amounts of blood meal. While these results are not in direct accord with those of the previous experiment, the general trend is none the less the same in both experiments.

The data in Table XXI show a marked uniformity in the results of all series with the exception of the series receiving 2 % manure. The difference in the other series are slight and indicate but little influence due to the manurial additions. In the previous experiment the series receiving manure showed a decreased rate of evaporation in the presence of bacteria. In the present experiment the 2 % manure series again shows the

TABLE XXI. INFLUENCE OF ADDITION OF MANURE

Day interval	Loss of moisture in grams		Difference in favor of inoc- ulated set	Total num- ber of days	Loss of moisture in grams		Difference in favor of inoc- ulated set
	Sterile	Inoc- ulated			Sterile	Inoc- ulated	
Series I No manure							
1st.....	19.3	19.5	+0.2	1	19.3	19.5	+0.2
2nd.....	16.5	14.6	-1.9	2	35.8	34.1	-1.7
3rd.....	16.5	15.2	-1.3	3	52.3	49.3	-3.0
4th.....	17.2	17.6	+0.4	4	69.5	66.9	-2.6
5th.....	19.2	21.3	+2.1	5	88.7	88.2	-0.5
6th & 7th.....	30.0	31.2	+1.2	7	118.7	119.4	+0.7
8th.....	15.5	19.2	+3.7	8	134.2	138.6	+4.4
9th.....	13.0	15.0	+2.0	9	147.2	153.6	+6.4
10th.....	17.6	19.6	+2.0	10	164.8	173.2	+8.4
11th.....	12.2	10.6	-1.6	11	177.0	183.8	+6.8
Series II +1% manure							
1st.....	17.6	18.6	+1.0	1	17.6	18.6	+1.0
2nd.....	15.6	15.2	-0.4	2	33.2	33.8	+0.6
3rd.....	17.0	16.6	-0.4	3	50.2	50.4	+0.2
4th.....	17.8	17.3	-0.5	4	68.0	67.7	-0.3
5th.....	20.3	20.8	+0.5	5	88.3	88.5	+0.2
6th & 7th.....	30.2	31.8	+1.6	7	118.5	120.3	+1.8
8th.....	15.0	16.3	+1.3	8	133.5	136.6	+3.1
9th.....	13.3	15.6	+2.3	9	146.8	152.2	+5.4
10th.....	18.0	19.2	+1.2	10	164.8	171.4	+6.6
11th.....	11.8	11.0	-0.8	11	176.6	183.4	+5.8
Series III +2% manure							
1st.....	18.0	17.0	-1.0	1	18.0	17.0	-1.0
2nd.....	16.0	15.8	-0.2	2	34.0	32.8	-1.2
3rd.....	17.2	16.2	-1.0	3	51.2	49.0	-2.2
4th.....	17.8	18.0	+0.2	4	69.0	67.0	-2.0
5th.....	22.0	20.6	-1.4	5	91.0	87.6	-3.4
6th & 7th.....	30.3	29.8	-0.5	7	121.3	117.4	-3.9
8th.....	16.5	17.3	+0.8	8	137.8	134.7	-3.1
9th.....	13.0	15.3	+2.3	9	150.8	150.0	-0.8
10th.....	18.0	18.0	0.0	10	168.8	168.0	-0.8
11th.....	9.5	9.7	+0.2	11	178.3	177.7	-0.6
Series IV +3.5% manure							
1st.....	16.6	17.6	+1.0	1	16.6	17.6	+1.0
2nd.....	17.6	17.0	-0.6	2	34.2	34.6	-0.4
3rd.....	16.2	16.6	+0.4	3	50.4	51.2	+0.8
4th.....	18.5	17.6	-0.9	4	68.9	68.8	-0.1
5th.....	20.2	19.0	-1.2	5	89.1	87.8	-1.3
6th & 7th.....	29.2	32.6	+3.4	7	118.3	120.4	+2.1
8th.....	14.5	15.5	+1.0	8	132.8	135.9	+3.1
9th.....	12.6	13.8	+1.2	9	145.4	149.7	+4.3
10th.....	15.8	16.3	+0.5	10	161.2	166.0	+4.8
11th.....	11.0	10.2	-0.8	11	172.2	176.2	+4.0
Series V +5% manure							
1st.....	19.8	16.2	-3.6	1	19.8	16.2	-3.6
2nd.....	17.0	16.5	-0.5	2	36.8	32.7	-4.1
3rd.....	15.3	17.0	+1.7	3	52.1	49.7	-2.4
4th.....	16.6	18.3	+1.7	4	68.7	68.0	-0.7
5th.....	19.3	21.8	+2.5	5	88.0	89.8	+1.8
6th & 7th.....	29.6	31.2	+1.6	7	117.6	121.0	+3.4
8th.....	14.3	16.2	+1.9	8	131.9	137.2	+5.3
9th.....	12.2	13.6	+1.4	9	144.1	150.8	+6.7
10th.....	15.2	16.6	+1.4	10	159.3	167.4	+8.1
11th.....	10.6	10.2	-0.4	11	169.9	177.6	+7.7

same decrease. Thus the results for both experiments with 2 % manure are similar and raise the question as to the causal factor concerned in producing such a reduction. It seems strange that the addition of 2 % manure alone should exert such an influence, whereas other amounts of manure show no such reduction. The complexity of the processes involved makes it difficult to offer an explanation. The fact remains that the presence of 2 % manure occasioned a reduction in the evaporation due to inoculation. No other instance of such a condition has been observed in any of the experiments performed.

#### PROBLEM VI. INFLUENCE OF A DRY ATMOSPHERE ON EVAPORATION

In the work thus far reported all experiments were conducted in the special room in which, however, it was impossible to control or keep constant the humidity of the air. The result was more or less variation in the moisture of the air resulting in a fluctuation in the rate of evaporation from day to day, being greater on a bright sunny day and less on a dark rainy day. One finds as a result, little uniformity in the daily evaporations in the experiments thus far performed. A series of plates was therefore arranged, so that a constant stream of air, dried by passing through sulfuric acid and calcium chloride was run over the exposed plates, the moisture taken up by this current of air being reabsorbed by sulfuric acid. The increase in weight of the sulfuric acid bottle was considered to indicate the moisture lost by evaporation. Owing to lack of sufficient apparatus, only one plate each (inoculated and sterile) could be run at a time. The method, however, did not prove so satisfactory as was at first thought; variations in the water pressure used to suck the air through the apparatus, occasioned marked differences in the daily losses due to evaporation. Thus at certain intervals the evaporation per day was 26 grams, whereas at other times it was less than 5 grams. This is evident from Table XXII. It is interesting to note, however, that here again the inoculated set exceeded the sterile in rate of evaporation. After several futile attempts to regulate the water pressure to give uniform results, the method was discarded as unsuitable for the work in hand.

TABLE XXII. EVAPORATION IN A DRY ATMOSPHERE

The air was dried by passing through sulfuric acid and calcium chloride.

Hour interval	Loss of moisture in grams		Increased evaporation in grams of inoculated set
	Sterile	Inoculated	
1st 12.....	5.1	5.4	0.3
2nd 12.....	4.5	4.7	0.2
24th to 60th.....	3.3	5.3	2.0
60th to 72nd.....	4.0	7.0	3.0
72nd to 120th.....	21.7	26.7	5.0
120th to 144th.....	9.1	10.3	1.2
Total.....	47.7	59.4	11.7

## PROBLEM VII. CAPILLARY ACTION OF THE SOIL MOISTURE

It is evident throughout that the presence of bacteria was instrumental in accelerating the rate of evaporation. It seems hardly probable that the bacteria themselves are directly responsible for the increased evaporation secured when they were present, in fact one would expect a retardation, if any influence at all, similar to that secured in the presence of the diluted gelatin solution. It is more probable that the marked bacterial activity which undoubtedly occurs in the normal or inoculated series, is accompanied by a pronounced metabolism with the production of soluble by-products of an inorganic as well as organic nature. These undoubtedly change the surface tension of the soil moisture, whereas, no such change occurs in the sterile controls. The concentration of the soil water is thus modified. Mineral salts usually increase the surface tension whereas soluble organic compounds especially those of an oily nature, decrease it. Whether the surface tension will be greater than that of pure water will depend upon the proportion of organic to mineral compounds in solution. Thus soil extracts usually show a much lower surface tension than pure water, although they contain dissolved salts. The soluble organic compounds dissolved in such extracts usually more than neutralize the effect of the mineral compounds, the result being a reduction in the surface tension.

If during the process of bacterial development, a conversion of organic to mineral compounds occurs, then one should expect an increase in the surface tension. It is reasonable to suppose that the processes of protein decomposition (ammonification and

nitrification) occur under conditions such as prevailed in the experiments, as well as that an increase in the concentration of the mineral constituents in the soil solution takes place as a result of the solvent action of the carbonated water produced by the bacterial metabolism. These two factors would tend to decrease the amount of soluble organic matter in the soil water and simultaneously to increase the concentration of the mineral constituents, which process would mean an increased surface tension.

Such an increase in the surface tension would mean an increase in capillary action. If capillary action is thus increased, the tendency would be to hold more water at the surface of the soil as well as to bring more there, than in the sterile plates where no such increased surface tension occurs. This exposure of more water to evaporation in the normal or inoculated series, would account for the difference in rate of evaporation between the inoculated and sterile series which has thus far been observed.

Proceeding on this basis the following experiment was arranged to determine the accuracy of the above explanation as to the cause of the increased rate of evaporation.

Percolators were used because they were open at both ends thus preventing the danger of any possible error due to gas formation, as pressure exerted by such gas would be equalized downward as well as upward. The upward pressure in vessels closed at the bottom is considered in a subsequent experiment. Three series of four percolators each were treated and arranged according to the plan presented in Table XXIII. In each series there were two normal or inoculated percolators and two others kept sterile by means of the weak mercuric chloride solution, being identical in all other respects.

The percolators were allowed to stand in a warm room to permit marked bacterial development. The contents of each percolator were then removed, air-dried, and mixed, separately. The dried and well mixed soils were then restored to their respective percolators, and 600 c. c. of sterile distilled water gradually added to the surface of each. The excess water running through was retained for several surface tension experiments which will be discussed later.

After remaining thus for three days, moisture determinations were made upon the top and bottom soil layers in each of the

TABLE XXIII. EFFECT OF CAPILLARITY ON MOISTURE AT SURFACE OF SOIL

Plan of experiment on influence of increased capillarity upon amount of moisture at the surface of soil columns.

Series	Condition	No. of percolator	Treatment
I. 1200 grams quartz sand	Normal ....	1 2	100 c. c. nutrient solution 200 c. c. soil infusion
	Sterile .....	1 2	100 c. c. nutrient solution 180 c. c. soil infusion+20 c. c. HgCl <sub>2</sub> solution
II. 900 grams clay loam	Normal ....	1 2	100 c. c. water 200 c. c. soil infusion
	Sterile .....	1 2	100 c. c. water 180 c. c. soil infusion+20 c. c. HgCl <sub>2</sub> solution
III. 900 grams muck	Normal ....	1 2	100 c. c. water 200 c. c. soil infusion
	Sterile .....	1 2	100 c. c. water 180 c. c. soil infusion+20 c. c. HgCl <sub>2</sub> solution.

percolators. If, on the basis of the explanation given above, increased capillary action existed in the inoculated series, then there should be a higher moisture content in the surface layers of the latter than in the sterile series. Consultation of the data in Table XXIV reveals such an increased moisture content in all cases, being greatest in the muck soil and least in the sand.

TABLE XXIV. MOISTURE CONTENT, BY WEIGHT, OF TOP AND BOTTOM SOIL LAYERS IN PERCOLATORS CONTAINING INOCULATED AND STERILE SOILS

Condition	Sand Per cent moisture		Clay loam Per cent moisture		Muck Per cent moisture	
	Top	Bottom	Top	Bottom	Top	Bottom
Inoculated.....	5.5	21.8	30.3	31.0	37.0	40.0
Sterile .....	3.5	21.8	27.8	31.0	30.5	40.0
Difference in favor of inoculated series.....	2.0	0.0	2.5	0.0	6.5	0.0

The figures in Table XXIV are the average of the two percolators in each set. In contrast to the difference in the top layers, one finds a marked uniformity in the moisture contents of the bottom layers for both inoculated and sterile series. It appears from this that more water is retained in the top layers of the inoculated series, and that this is due to greater capillary action. The downward pull of gravity is opposed by the upward force of capillarity more in the inoculated series than in the sterile series. These results thus apparently substantiate the statement that the development of the soil bacteria occasions a change in the surface tension of the soil water due to changes in the composition and proportion of soluble compounds contained therein; this change in surface tension causes greater capillary action which means that more water is retained in, and brought to, the surface layers and exposed to evaporation. Why muck should exceed the clay loam and the sand, can perhaps be explained on the basis of the fineness of the soil particles. It is known that the finer the soil particles, the greater the number of capillary spaces, which in turn increases the capillary pressure. Of the soils used in the experiments the muck was the finest grained. No doubt the greater amount of insoluble organic matter in the muck also served as a contributing factor in producing the increased difference.

The filtrates obtained from the percolator experiment were employed to determine their capillary rise in sand tubes of ordinary glass tubing 1.2 cm. in diameter and 500 cm. long. Each tube was sealed at the lower end with a cheese cloth cap, and filled with quartz sand which had been passed through an 80-mesh sieve. The tubes were then set in beakers containing the various filtrates and the rates at which the latter ascended by capillarity in the sand columns noted. Filtrates from the inoculated soils rose faster than those from the sterile soils, which further substantiates the explanation to account for the increased evaporation in the presence of bacteria; namely that they cause an increased capillarity.

#### PROBLEM VIII. INFLUENCE OF GAS FORMATION ON EVAPORATION OF MOISTURE

The preceding experiments have shown that there is a greater capillary rise of soil water in all cases where bacterial develop-



ment occurs. Evidently in the process of bacterial multiplication, by-products are produced which increase the surface tension of the soil water. This occasions a greater capillary action resulting in a movement of the soil water to the surface, where it is exposed to evaporation. Whether this is the only factor contributing as a cause of the increased evaporation where bacterial multiplication occurs, is not known but there are probably other contributory agencies.

It is a well-known fact that under certain conditions, gas forms in soil. This is particularly true where there are large quantities of decaying organic matter in the presence of excessive moisture, as under waterlogged conditions in swamps and marshes. Here one frequently has marked evolution of methane. The production of such gases in the lower soil layers is undoubtedly instrumental in bringing the moisture from the lower soil layers to the surface, especially where a condition of saturation prevails. While the soil water has a continuous unbroken passage to the surface by reason of its capillary film, the gas generated has not. The result is, that the gas in endeavoring to escape from the lower soil layers, must force the soil water in advance of it, and thus brings the water to the surface. That this phenomenon can occur, was well demonstrated in the following manner. Several large glass cylinders were filled with quartz sand to a depth of 14 inches. To these were then added enough dilute 1% dextrose bouillon to saturate the sand columns. Four cylinders each received 500 c. c. of the bouillon and 100 c. c. of a soil infusion. Four others each received a similar amount of the bouillon and 100 c. c. of a soil infusion containing mercuric chloride. As the same amount of sand had been employed in all cylinders, all had received identical treatment except the addition of the mercuric chloride to one set of four. This set, of course, remained sterile.

After preparation all cylinders were incubated and the changes taking place, observed. After 24 hours the inoculated set showed signs of gas formation and a decidedly moist surface in contrast to the sterile set which revealed no gas formation, and which were practically dry at the surface. After 36 hours there was an accumulation of water on the surface of the inoculated set, brought there by the gas in the lower layers endeavoring to escape upward. The appearance of the cylinders at the

end of 48 hours is shown in Fig. 1. Whereas the surface of the sterile set was perfectly dry, that of the inoculated was covered with 1 to 2 inches of water, although both sets possessed the same initial moisture content.

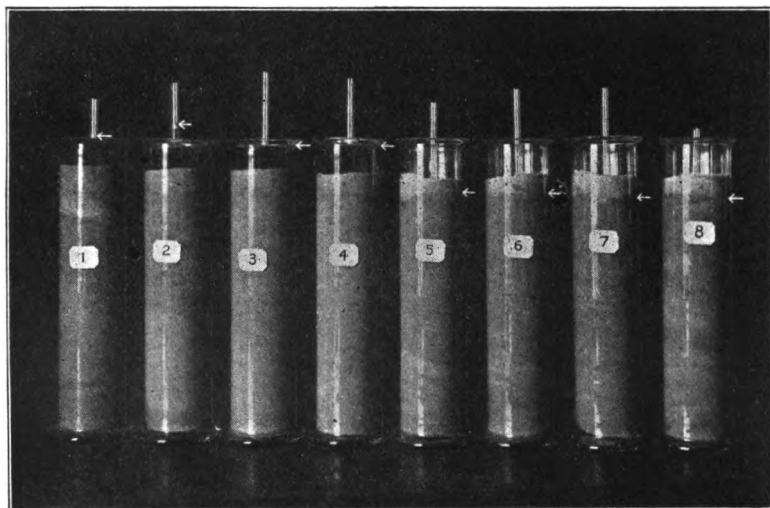


FIGURE 1. WATER FORCED TO SURFACE BY GAS

Influence of gas formation in forcing soil moisture to surface. Arrow points show surface of water.

Cylinders 1, 2, 3, and 4 were inoculated. Note water above sand.

Cylinders 5, 6, 7, and 8 were kept sterile. Note that surface of sand is dry.

#### PROBLEM IX. INFLUENCE OF PURE CULTURES UPON EVAPORATION

Supplementary to the preceding work, all of which was performed with a mixed soil bacterial flora, several experiments were conducted employing pure cultures of various organisms to ascertain whether the same increased evaporation could be secured under such conditions.

For this purpose two experiments were performed, one with cultures of *azotobacter* and the other with *Bacillus subtilis*. The general technique used in the preparation of the plates was the same as under the normal soil bacterial flora work, with the one exception that all the soils and sand were sterilized before inoculation. The substratum material, whether sand or soil, was placed in the plates in the desired quantities and sterilized in a dry condition in the autoclave. Suspensions of the organ-

isms were made in sterile distilled water, being made as uniform as possible by vigorous shaking with sand. After settling, the supernatant suspension was removed in two portions. To one of these mercuric chloride solution was added; to the other, an equal amount of sterile distilled water. The suspensions thus obtained, the one sterile, the other containing living bacteria,

TABLE XXV. INFLUENCE OF INOCULATION WITH *Bacillus Subtilis* ON EVAPORATION

Clay loam soil, garden soil, and quartz sand were used as substrata.

Day interval	Loss of moisture in grams		Difference in favor of inoculated set	Total number of days	Loss of moisture in grams		Difference in favor of inoculated set
	Sterile	Inoculated			Sterile	Inoculated	
Clay loam soil							
1st.....	28.72	24.95	-3.77	1	28.72	24.95	-3.77
2nd.....	13.45	14.77	+1.32	2	42.10	39.70	-2.40
3rd.....	18.35	21.20	+2.85	3	60.45	60.90	+0.45
4th.....	15.50	17.70	+2.20	4	76.00	78.65	+2.65
5th.....	15.70	16.90	+1.20	5	91.77	95.55	+3.78
6th.....	14.40	15.35	+0.95	6	106.17	110.90	+4.77
7th.....	12.55	13.77	+1.22	7	118.75	124.60	+5.85
8th.....	12.42	13.10	+0.68	8	131.15	137.70	+6.55
White quartz sand							
1st.....	29.70	26.77	-2.93	1	29.70	26.77	-2.93
2nd.....	14.65	16.32	+1.67	2	44.35	43.09	-1.26
3rd.....	18.25	20.17	+1.92	3	62.60	63.26	+0.66
4th.....	15.47	16.77	+1.30	4	78.07	80.03	+2.06
5th.....	18.55	18.40	-0.15	5	96.62	98.43	+1.81
6th.....	17.10	17.65	+0.55	6	113.72	116.08	+2.36
7th.....	15.60	17.77	+2.17	7	129.32	133.85	+4.53
8th.....	14.70	15.65	+0.95	8	144.02	149.50	+4.48
Garden soil							
1st & 2nd.....	31.8	30.5	-1.3	2	31.8	30.5	-1.3
3rd.....	17.0	21.1	+4.1	3	48.8	51.6	+2.8
3rd to 5th.....	26.1	32.1	+6.0	5	74.9	83.7	+8.8
5th to 7th.....	37.3	34.9	-2.4	7	112.2	118.6	+6.4
7th to 9th.....	28.7	30.6	+1.9	9	140.9	149.2	+8.3

were then employed to moisten the sterile sand or soil in the plates. The same amounts of liquid were added to all plates. Thus all received identical treatment with the one exception of the addition of mercuric chloride to the sterile sets.

Weighings were made as usual, the losses in weight from day to day being recorded as due to evaporation. Several soils and quartz sand were employed as substrata. The results of these experiments are recorded in Tables XXV and XXVI.

One finds here under pure culture conditions the same relationship existing between sterile and inoculated series as with the mixed bacterial inoculation of the earlier experiments. In all cases there is an increased rate of evaporation in the inoculated series. This increase was greater in the case of azotobacter than where *Bacillus subtilis* was employed, which is strange,

TABLE XXVI. INFLUENCE OF INOCULATION WITH AZOTOBACTER ON EVAPORATION

Garden soil, field soil, and quartz sand were used as substrata.

Day interval	Loss of moisture in grams		Difference in favor of inocu- lated set	Total num- ber of days	Loss of moisture in grams		Difference in favor of inocu- lated set
	Sterile	Inocu- lated			Sterile	Inocu- lated	
Garden soil							
1st & 2nd .....	31.8	37.9	+ 6.1	2	31.8	37.9	+ 6.1
3rd .....	17.0	21.8	+ 4.8	3	48.8	59.7	+10.9
3rd to 5th .....	26.1	36.2	+10.1	5	74.9	96.0	+21.1
5th to 7th .....	37.3	38.5	+ 1.2	7	112.2	134.5	+22.3
7th to 9th .....	28.7	31.5	+ 2.8	9	140.9	166.1	+25.2
Field soil							
1st .....	19.5	20.2	+ 0.7	1	19.5	20.2	+ 0.7
2nd .....	19.4	20.7	+ 1.3	2	38.9	40.9	+ 2.0
3rd .....	16.3	19.8	+ 3.5	3	55.2	60.7	+ 5.5
4th .....	15.5	19.0	+ 3.5	4	70.7	79.7	+ 9.0
5th .....	18.3	20.2	+ 1.9	5	89.0	99.9	+10.9
6th .....	17.9	20.4	+ 2.5	6	106.9	120.3	+13.4
7th .....	18.4	23.0	+ 4.6	7	125.3	143.3	+18.0
8th .....	19.1	21.5	+ 2.4	8	144.4	164.8	+20.4
9th .....	15.2	17.9	+ 2.7	9	159.6	182.7	+23.1
10th .....	13.3	16.1	+ 2.8	10	172.9	198.8	+25.9
11th .....	11.5	12.3	+ 0.8	11	184.4	211.1	+26.7
12th .....	8.0	7.9	- 0.1	12	192.4	219.0	+26.6
13th .....	7.8	7.5	- 0.3	13	200.2	226.5	+26.3
Quartz sand							
1st .....	22.0	22.3	+ 0.3	1	22.0	22.3	+ 0.3
2nd .....	20.9	21.8	+ 0.9	2	42.9	44.1	+ 1.2
3rd .....	18.1	19.6	+ 1.5	3	61.0	63.7	+ 2.7
4th .....	18.6	20.3	+ 1.7	4	79.6	84.0	+ 4.4
5th .....	19.9	21.8	+ 1.9	5	99.5	105.8	+ 6.3
6th .....	19.7	23.1	+ 3.4	6	119.2	128.9	+ 9.7
7th .....	23.5	25.8	+ 2.3	7	142.7	154.7	+12.0
8th .....	21.0	24.0	+ 3.0	8	163.7	178.7	+15.0
9th .....	16.0	20.3	+ 4.3	9	179.7	199.0	+19.3
10th .....	11.7	15.3	+ 3.6	10	191.4	214.3	+22.9
11th .....	8.5	9.5	+ 1.0	11	199.9	223.8	+23.9
12th .....	5.1	2.8	- 2.0	12	205.0	226.6	+21.6
13th .....	3.8	0.5	- 3.3	13	208.8	221.1	+18.3

as with azotobacter one has the production of a more or less slimy zoogloeal mass which on drying assumes a tough gelatinous consistency in which form one would expect it to hinder evaporation. Instead, one finds a very marked acceleration, probably due to the vigorous growth which azotobacter made under the conditions of the experiments. The work on the pure cultures thus further substantiates the previous work and

strengthens the conclusion drawn, namely that the presence of bacteria increases the rate of evaporation from the soil.

### SUMMARY

Any conclusions which can be drawn from this work must be considered with great care to avoid any erroneous deductions or applications. It must be remembered that the conditions prevailing in the experiments only approximate field conditions. It is believed that the phenomena here observed do exert an influence under field conditions. The movement of soil water is known to be dependent upon such forces as gravitation, surface tension, capillarity, temperature, and viscosity, as well as upon the chemical and physical composition of the soil itself, but to these must be added the biological activities taking place in the soil.

Changes in the quantity and quality of the various chemical substances in solution in the soil water result from bacterial activities. These changes probably disturb the equilibrium of the soil moisture so far as its distribution is concerned, and help to cause its movement and circulation.

The principal effect of the bacterial activities is undoubtedly upon the surface tension of the soil moisture. Any change produced in this, means a diffusion and a movement of the soil water from the point of lower tension to that of higher tension, in an endeavor to readjust itself uniformly throughout the soil mass; in other words capillary action will come into play. Thus, if through the addition of soluble mineral salts, artificially or as a result of the conversion of insoluble substances to soluble compounds in the process of protein decomposition and mineralization, the surface tension of the water in the upper layers is increased there will result an upward movement to that point. This is probably what occurred in the percolator experiment where the upper soil layers of the inoculated series showed a higher moisture content than the corresponding soil layers in the sterile series.

Again if there are substances present which possess the property of occasioning a reduction in surface tension such as alcohol produces in water, and which are formed as by-products in the decomposition of certain protein or carbonaceous materials, a reduction in surface tension occurs, and a movement away from

the surface results. It is possible that such a condition occurred in the manure experiment, the one instance where the inoculated series showed a less rapid rate of evaporation than the sterile series.

The production of carbon dioxide by bacteria in soil is another factor which undoubtedly increases the surface tension. This may be considerable, amounting in some instances as Stoklasa<sup>4</sup> has shown, to 1.5 liters per kilogram of soil per year. The carbon dioxide thus formed is dissolved in the soil water, producing an acid reaction, increasing thereby more or less markedly the solvent action of the soil water upon inorganic mineral compounds with which it comes in contact. The increased solution of such mineral substances undoubtedly increases the surface tension and results in a movement of the soil water.

The production of gaseous compounds in the lower soil layers is not a frequent occurrence, as the conditions necessary for such are found only in low, swampy places, marshes, etc., where a waterlogged condition is combined with the presence of large amounts of organic matter. Here gas production such as occurred in the experiment with the glass cylinders may produce a similar condition and tend to prevent the percolation of the water through the soil, keeping it on the surface. One would not expect gas formation under other natural conditions and accordingly little stress can be laid upon the factor of gas formation as an agent in causing the increased evaporation observed.

In general then, it would appear from the experiments that the soil bacteria and their activities are factors which must be considered when discussing the movement of soil water; not so much because of the cells themselves as because of the by-products which they form and the subsequent influence of the same upon such factors as surface tension, capillarity, viscosity, etc. of the soil moisture. The biological feature of the soil apparently forms an important contributory factor in determining the movement of soil water.

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<sup>4</sup> Centbl. Bakt., Abt. II, 29, 1911, p. 409.



# The Diagnosis of Contagious Abortion in Cattle by Means of the Complement Fixation Test

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F. B. HADLEY and B. A. BEACH .

The importance of contagious abortion from the economic viewpoint can not be overestimated. The losses incident thereto usually extend over a number of years with each infected animal. Not only is the calf lost, due to its premature expulsion from the womb, but the milk yield of the cow is materially reduced; she frequently fails to conceive, and her productivity thus becomes impaired for varying lengths of time.

The disease exists in nearly all sections of the world where dairying is engaged in to any extent. It is more prevalent in northern portions of the United States, on account of the greater development of the dairy industry, than in the southern parts. Well informed men maintain that very few breeders who have been in the business six or eight years can truthfully say they have not experienced trouble with the disease. For these reasons, live stock producers should be interested in the different aspects of this highly important and extensively distributed disease.

Contagious abortion is most frequently seen in the bovine species, and is caused by a specific microorganism which finds the pregnant uterus a particularly favorable location for growth. It is usually characterized by the expulsion of the fetus before the period of gestation has been completed.

## HISTORICAL REFERENCES

Until a few years ago it was thought that contagious abortion was caused by a number of different organisms; that none of them were specific to the exclusion of the others.



The Scottish Commission<sup>1</sup> found as many as five different organisms in the genital tract of aborting cows. In France, Nocard<sup>2</sup> found coccus-like and very delicate rod-shaped organisms which lived in the womb throughout the interval between pregnancies.

By far the most important of all the contributions to this subject is that of Professor Bang<sup>3</sup> who in 1895 undertook the investigation of this disease. He found many very small bacilli in stained preparations of the uterine exudate between the fetal membranes and the wall of the uterus of a cow which had shown signs of abortion. This investigator was able to cultivate this organism in pure culture on gelatin-agar-serum, and also to produce abortion experimentally and recover the specific organism.

Nowak<sup>4</sup> in Austria made gelatin-agar-serum plates, allowed them to solidify, after which they were streaked with material from a suspected subject and placed in a sealed jar containing culture of *Bacillus subtilis* to exhaust the oxygen, one square inch of culture surface to every 15 c. c. jar capacity.

In this country Dr. W. J. MacNeal,<sup>5</sup> formerly of the Illinois Experiment Station, Prof. E. S. Good<sup>6</sup> of the Kentucky Experiment Station, and other investigators have succeeded in isolating an organism which they believe to be the Bang bacillus.

#### ETIOLOGY

The causal agent in contagious abortion of cows has been named the *Bacillus abortus* (Bang). It is a cocco-bacillus 0.8 to 2 microns long by 0.5 to 0.7 wide. The organism stains with the aniline dyes and is Gram negative. When carbol fuchsin is used, it appears much larger than when stained with methylene blue or gentian violet. Microscopical examination will reveal a very small coccus-like organism which may easily be mistaken for the ordinary pus cocci. Close scrutiny, however, will show

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<sup>1</sup> Woodhead, McFadyean & Aitkin. Report of the Scottish Commission.

<sup>2</sup> Nocard, Review by Bang. Zeitschrift für Thiermed. I. 1897.

<sup>3</sup> Bang. Die Aetiologie des seuchenhaften ("infectiösen") Verwerdens. Zeitschrift für Thiermed. I. 1897.

<sup>4</sup> Nowak. Le bacille de Bang et sa biologie. Annales de l'Institut Pasteur. Vol. 22; pp. 541-546. 1908.

<sup>5</sup> McNeal, W. J. and Mumford, H. W., Ill. Bul. 152. 1911.

<sup>6</sup> Good, E. S. The Etiology of Contagious Abortion. Am. Vet. Rev. Vol. XL, No. 4. Jan. 1912.

that some of the organisms have a slightly elongated appearance, the more striking if some micrococcus is observed at the same time.

Blood-serum-agar or bouillon has been found the best medium for the isolation and growth of the abortion bacillus. (See Figures 1 and 2). It is usually necessary to grow a strain recently isolated in a rarefied atmosphere or in a tension of oxygen somewhat greater than one atmosphere. Growth under these conditions takes three to four days. The behavior towards oxygen and the fact that growth is slight or does not occur at all on ordinary culture media are valuable means of identification.

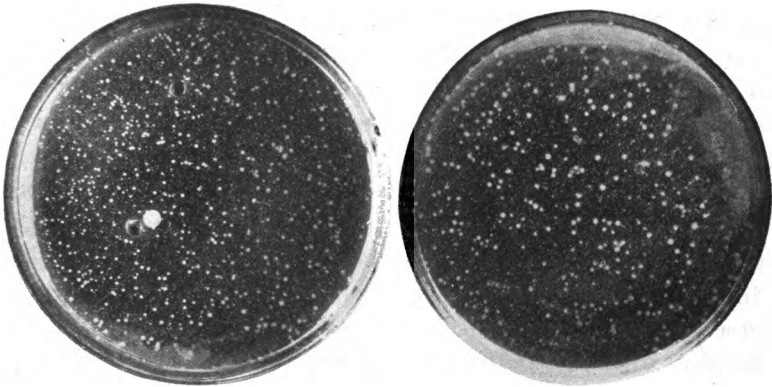


FIGURE 1. HORSE SERUM-AGAR

FIGURE 2. OX SERUM-AGAR

Serum-agar plates of *Bacillus abortus* after seventy-two hours incubation. The inoculations were made directly from the stomach contents of an aborted fetal calf. Note the larger, more luxuriant growth on the ox serum-agar.

After a strain has been transferred several times, it will usually grow fairly well under atmospheric conditions on blood-serum media. Some strains seem to acquire the faculty of growing on ordinary media; others do not. One strain which was isolated grew under ordinary atmospheric conditions immediately. It would also grow slightly on plain agar. The colonies are round, slightly convex, smooth, and translucent; simulating a honey- or dew-drop. When observed by transmitted light a characteristic bluish cast is noticeable.

Bang and Stribolt demonstrated that there were two optima as regards oxygen tension. With the gelatin-agar-serum in pure oxygen the growth developed best in two zones; one near the surface, the other near the bottom of the tube. They concluded

that it was neither a strict aerobe nor an anaerobe, but that it required a tension of oxygen either somewhat greater than one atmosphere or a little less.

Microscopic examination will often reveal a small organism in the uterine exudate of a cow which has recently aborted, in the scrapings of the fetal membranes, the umbilicus, and in the stomach contents of the aborted fetus. Mohler<sup>7</sup> calls attention to the experiment of Schroeder and Cotton<sup>8</sup> in which micro-organisms similar to the abortion bacilli were found in guinea pigs that had been injected with milk from infected cows.

The plate method of isolation is used altogether. Ordinary plain agar is heated until it liquefies, when it is cooled to about 50° C. Naturally sterile blood serum is added, approximately 1 c. c. of serum to every 5 c. c. of agar. The tubes are kept at the above temperature. The abdominal cavity of the fetus is opened, revealing the stomach, the wall of which is seared with a hot iron, after which it is incised with a sterile scalpel. A loopful of the stomach contents is at once transferred to a tube of the medium, a dilution tube is inoculated from the first and the contents of both are immediately poured into sterile petri dishes. When the medium has solidified the plates are placed in a jar containing *Bacillus subtilis* after the method of Nowak. In place of the *B. subtilis* the air may be exhausted to 200 mm. by use of an ordinary vacuum pump. An examination is made after four to five days' incubation.

#### CLINICAL FEATURES

A bulletin of this nature, in which but one phase of the contagious abortion problem is considered in detail, must limit reference to the clinical aspects of the disease. Therefore, many interesting and important points relative to the pathology, symptoms, treatment, etc., will necessarily be omitted. However, before taking up the diagnostic methods with which our experiments have been particularly concerned, it may be well to call attention to a few facts which will aid the reader to understand the nature of the malady.

<sup>7</sup> Bur. Anim. Indus. Cir. 198. 1912.

<sup>8</sup> Schroeder, E. C. and Cotton, W. E. Proc. Am. Med. Vet. Assoc. pp. 195-206. 1911

The principal pathological lesions produced as a result of infection with the abortion bacilli occur in the pregnant uterus and its contents. When present in large numbers the organisms appear to set up an inflammation in the fetal membranes, especially at the points of union with the maternal membranes, which in the cow are known as cotyledons. These finally become involved to such an extent that the natural exchange of gases and nutrients can no longer take place between the mother and the fetus with the result that abortion or premature birth usually occurs. The placental membranes sometimes become abnormally thickened and inflamed, due to the irritation of the bacilli. Possibly certain toxins, the products of bacterial growth, are influential in the inflammatory changes, but further researches must be made along this line before this supposition is proved. Frequently a peculiar granular or nodular appearance of the placenta is to be noted as the result of this infection. Many veterinary practitioners regard this condition as pathognomonic of contagious abortion.

Infection may gain entrance to the body by a number of different routes. The genital passage is a frequent avenue for the entrance of the bacilli. Experiments made by the British Commission<sup>9</sup> indicate that the mouth must be considered an important portal for the entry of these disease producing organisms. Artificial infection with natural material and cultures of the abortion bacillus by subcutaneous and intravenous inoculations have also been demonstrated to be methods by which the cow could be caused to abort.

The discharges from the uterus of a diseased cow are probably the chief source of infection. Not only are they dangerous before and at the time of abortion, but also for an indefinite period thereafter. There is evidence to support the belief that occasional cases may act as carriers of the abortion bacilli for many months after the last abortion, as do the so-called typhoid fever carriers of the human race.

It is commonly held that the bull may convey the infection. Undoubtedly a bull may, after serving an infected cow, carry on his copulatory organ infectious material to the cows he subsequently serves so that they too become infected. However, at-

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<sup>9</sup> Report of Departmental Committee to Inquire into Epizootic Abortion. Appendix to Part 1. London, 1909.

tempts to infect heifers in this way have usually been unsuccessful.

The symptoms exhibited in the early months of pregnancy by a cow about to abort are frequently so slight as to pass unnoticed. The incompletely developed fetus is expelled while the cow is at pasture, or in the stable at night, and is so small that it may escape the attention of the attendant. Aside from the slight discharge from the vagina and swelling of the udder, the animal may show no evidences of abortion. Later in gestation, a marked enlargement of the udder, swelling of the external genital organs, and a vaginal discharge will be noted, together with the other symptoms which usually accompany a normal parturition.

Under ordinary farm conditions abortion occurs from the second to the seventh month of pregnancy. Fetuses expelled later than this, if they live, should rightly be called premature births, but usage has stretched the term abortion to include any birth before the normal term of gestation is completed.

Our knowledge so far as the treatment goes is very limited; most medicinal agents now employed are used empirically and have no scientific basis. For these reasons no attempt will be made to discuss the subject of therapeutics at this time.

#### HANDLING INFECTED ANIMALS

Because no effective curative treatment is at hand and on account of the facility with which abortion can now be diagnosed, methods of prevention will be considered more in detail. The most emphasis must be placed upon the great importance of properly disposing of the placentae, fetuses, and contaminated litter, especially as the abortion bacilli have been found to retain their vitality for a considerable time outside the body.

The following preventive measures may be suggested: Remove and bury infectious material deeply or burn it. Disinfect the gutters, floors, and walls with a 1-1000 solution of corrosive sublimate. Avoid buying cows from a district where abortion is known to exist. Unless the complement fixation test can be applied when new cows are purchased, it is well to quarantine such animals until they calve. The vulva, tail, thighs, and udder of each cow in an infected herd should be washed, sponged, or sprayed with a disinfectant solution each day. Those cows

which abort should be irrigated with a disinfectant douche. This douche can not be used in sufficient strength to destroy the germs on account of the irritating effects upon the mucous membrane, but it will aid in the prompt removal of the infectious organisms. In our opinion, the irrigation should be made at least once a day and continued until all evidence of the discharge has ceased. A 0.5 to 1% solution of any reliable coal tar disinfectant, or a 1-2000 solution of potassium permanganate may be used for this purpose. Cows in oestrus are more susceptible to irritants. In these cases a 3% solution of boric acid or bicarbonate of soda may be substituted. Bulls which have recently served infected cows should be disinfected by flushing out the sheath before another cow is covered. If an animal shows premonitory symptoms of abortion she should be immediately removed from the herd.

The best way to handle cows which have aborted, also those which have given a positive reaction to the complement fixation test, is to isolate them from the noninfected animals. As already pointed out, there is ample reason to believe that certain cows harbor the infectious organism for a considerable period after the abortion, otherwise there would be no plausible explanation for the recurrence of the disease in these animals the following year. Such cows are to be regarded as possible sources of infection and should not be stabled with noninfected cattle. Many infected cows will not readily conceive after infection, in fact some remain barren permanently and soon become nonsupporting. For these reasons it is better to either dispose of these animals for beef purposes, or maintain a separate herd, stable, and pasture.

The policy adopted at this station is to test all cattle in the herds each year. Any new animals proposed to be introduced are tested before they are placed with the regular herd. By these means, it is expected that the disease will be prevented here.

It is well known that a certain degree of immunity is conferred by an attack of abortion. In one dairy herd composed of 700 animals, where careful records were kept, 133 aborted in a period of three years. Of these aborters which again conceived, 28.6% aborted a second time, 14.2% the third time, thus leav-

ing 57.2% which aborted but once. Many of the last mentioned must therefore have acquired an immunity.

Recently the statement has been made that a herd of cows which had aborted would be more valuable for breeding purposes, on account of the acquired immunity, than a stock that had never been exposed to the infection. This would probably be true in a locality where the disease was epizootic, but would only be a temporary asset, as a large herd of this kind would undoubtedly contain certain cows which, although immune themselves, would harbor the disease producing organisms. Such cows would be capable of transmitting the infection not only to all newly purchased cattle, but also to the calves from the immune cows which, unfortunately, are not always rendered congenitally immune. Besides, a breeder who makes a practice of raising pure bred stock for breeding purposes would soon gain a bad reputation if he were known to maintain such a herd.

#### METHODS OF DIAGNOSING CONTAGIOUS ABORTION

Among the various methods devised for the diagnosis of contagious abortion may be mentioned: the agglutination test; the reaction method; a bacteriological examination; and the complement fixation test.

*The Agglutination Test.* This test has been used by various investigators but by itself has not been found uniformly satisfactory. One reason for this is that an increase in the agglutinating power of sera from cows which have recently become infected does not occur for some time. Experiments seem to show that a diagnosis based upon the agglutination method alone, is only fairly reliable at best, but in a combination with other methods it will be found serviceable.

*The Reaction Method.* This method is based upon the success attained by the use of tuberculin and mallein, and consists of the subcutaneous injection of an agent prepared in a manner similar to that used in the manufacture of these diagnostic agents. McFadyean and Stockman have called this product "abortin."<sup>10</sup> Their experiments with it were confined to a relatively small number of animals. The results were not satisfactory enough to warrant any definite statement as to its value.

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<sup>10</sup> Report of Departmental Committee to Inquire into Epizootic Abortion. Appendix to Part I. London, 1909.

*Bacteriological Examination.* This method of diagnosis involves a microscopical and cultural examination of the placental membranes, the fetus, and the uterine exudate. Stained slide preparations are made from scrapings taken from the cotyledons, and other parts of the placenta, the stomach fluid of the fetus, and from the uterine exudate. When the abortion bacilli are present in large numbers, they may be demonstrated in such preparations. The most satisfactory results are obtained, however, by making cultural examinations of fresh material on serum-agar medium. The characteristic honey- or dew-drop-like colonies will appear after three to five days incubation under the proper oxygen tension.

*The Complement Fixation Test.* Certain infectious diseases, such as syphilis and glanders have recently been brought under better control by this remarkable test which was introduced to the attention of scientists by Bordet and Gengou. It is based upon the hemolytic action of a specially prepared blood serum used in connection with blood sera of other species of animals.

Use has lately been made of this test in the diagnosis of contagious abortion by a number of European investigators, particularly Holth<sup>11</sup> and Wall,<sup>12</sup> but so far as we know nothing has yet been published as to any extensive work done in this country.

To demonstrate the practical application of the test, we have carried out more than 500 examinations on sera from animals in a number of widely separated herds, which are kept under various conditions and may therefore be taken as fairly representative of the Wisconsin dairy industry.

The test is in reality quite complicated and can be performed only in a properly equipped laboratory. However, a skilled technician can easily test 50 or more animals a day. No more time is therefore consumed than in testing a like number of cattle for tuberculosis.

For complete details concerning the theories involved in the interesting phenomenon of hemolysis, which underlies the foundation of the test, refer to some of the original articles by Ehr-

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<sup>11</sup> Holth, H. Untersuchungen über die Biologie des Abortusbazillus und die Immunitätsverhältnisse des infectiösen Abortus der Rinder. Zeitschrift für Infektionskrankheiten der Haustiere. Band 10, Heft 4/5. 1911.

<sup>12</sup> Wall, S. Über die Feststellung des seuchenhaften Abortus beim Rinde durch Agglutination und Komplementbindung. Zeitschrift für Infektionskrankheiten der Haustiere. Band 10, Heft 1/2/3. 1911.



lich, Morgenroth, Sachs<sup>13</sup> and others. Only such fundamental points will be here considered as will enable one to understand the relationship of the different components used in the complement fixation test as applied to contagious abortion.

#### COMPONENTS USED IN THE COMPLEMENT FIXATION TEST

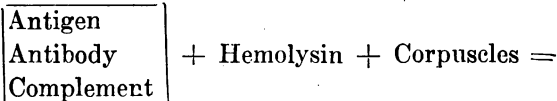
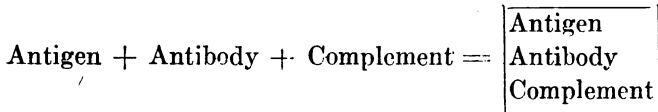
Five separate and distinct factors with a definite relationship to one another are required, as follows: (1) the suspect's blood serum; (2) antigen or prepared cultures of the abortion bacillus; (3) complement or fresh guinea pig blood serum; (4) hemolysin or blood serum from a specially treated rabbit; (5) washed red horse blood corpuscles.

When these substances are mixed in measured quantities and under proper conditions they may unite in different ways. One possible combination is the fixation of the complement by the antigen and antibody; another is a union of the complement and corpuscles by means of the hemolysin, resulting in hemolysis.

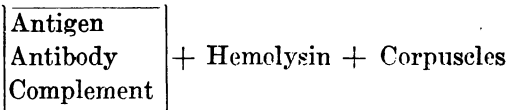
It is readily seen that the components must be used in definite quantities, for example, if an excess of the complement is used in a certain test in which the antigen and antibody are present in proper amounts, a portion is left free to act with the hemolysin and corpuscles to bring about hemolysis—an erroneous result.

The reaction may be graphically illustrated in the case of a positive serum, containing the specific antibodies, as follows:

(After first incubation, complement is fixed)



(After further incubation, no hemolysis)



<sup>13</sup> Ehrlich, Paul. *Studies in Immunity*. New York, 1910.

No hemolysis takes place here because the complement has been fixed or bound at the first incubation and so is not available to act with the hemolysin on the corpuscles.

In the case of a negative reaction where no antibodies exist in the serum the results would be as follows:

(After first incubation, no fixation)

Antigen + Serum + Complement = Antigen + Serum + Complement

Antigen + Serum + Complement + Hemolysin + Corpuscles

(After further incubation, hemolysis)

cles = Antigen + Serum + 

Corpuscles
Hemolysin
Complement

Hemolysis occurs in this reaction because the complement is not fixed and is therefore free to act at the second incubation.

Each of the factors will be described separately, in the order in which they are employed in the test; the method of standardization will be explained; and finally they will be brought together in proper quantities and manner to illustrate the technique followed in performing the test.

*The Suspect's Serum.* The blood of the animal to be tested is drawn from the jugular vein by means of a medium sized hypodermic needle or a capillary trochar. Easily excited animals may be conveniently controlled with a bull lead. Pressure with the thumb over the jugular will cause the vein to engorge sufficiently so that a stream of blood will flow from the canula. If many blood samples are required at one time, a small rope drawn tightly about the neck with a knot arranged to press upon the lower portion of the jugular, will help in this operation. The needles are kept in a large mouthed bottle containing a 1% solution of lysol. Lysol is more desirable than alcohol in that it does not coagulate blood so readily, and the needles are therefore easier cleaned.

The blood is caught in sterile test tubes holding about 10 c. c., or in large mouthed sterile bottles holding about 1 ounce. Each tube or bottle is labeled and properly corked. The advantage of test tubes is that they may be placed in a centrifugal machine later, if necessary, to separate the serum from the blood clot. Our practice has been to loosen the clot from the sides of the

tubes and place them in the ice box or other cool place over night to permit the serum to separate. The following morning the serum is ready for removal to smaller test tubes. Finely drawn pipettes to which a small rubber nipple is fitted will be found useful for this purpose. These smaller test tubes are convenient to handle the serum in while it is undergoing the process of inactivation.

By inactivation is meant the exposure of the serum to a temperature from 55° C. to 56° C. for one-half hour in a water bath. This procedure destroys the complement of the cattle serum. Complement, as will be more fully explained later, is a thermo-labile substance present in all normal sera but in varying amounts. Its destruction is necessary in this serum so that no interference may be experienced in the test.

If it is necessary to hold the serum for some days before testing, it should be drawn off the corpuscles, and preserved by adding to each 9 c. c., 1 c. c. of a solution consisting of 5 parts carbolio acid, 10 parts glycerin, and 85 parts of a physiological sodium chloride solution (0.9%). Stored in an ice box such carbolized serum should retain unchanged for several weeks, any immune bodies it may contain.

*The Antigen.* In the complement fixation test for abortion, the antigen used is an emulsion of the abortion bacilli or an extract of the same. This may be prepared in several different ways. (For description of different methods see page 245.) The method found most satisfactory is as follows: The organisms are grown on a serum-agar slope until a heavy growth is secured. This culture is washed off with 5 to 15 c. c. of salt solution per tube, depending on the amount of growth. It is then preserved by adding 10% of the aforementioned phenol-glycerin-salt solution, and shaken in a shaking apparatus for 24 hours. The best results have been obtained with a polyvalent antigen, i. e. one made with a number of strains of *B. abortus*. Such prepared antigens may be kept for several months if stored in a cool, dark place. They must, however, be retitrated from time to time to establish their strength. Anti-complements may form in old antigen; these may be readily destroyed by heating in a water bath at 56° C. for 20 minutes.

*The Complement.* Fresh blood sera from practically all animals contain complement in varying quantities. On this ac-

count care must be exercised to guard the various fluids used in the work against possible contamination with extraneous serum. Guinea pigs are best adapted to furnish complement on account of the more constant and very active complemental qualities of their serum. Only a small quantity is required in conjunction with the hemolysin to bring about hemolysis.

The blood is obtained by anesthetizing the animal with chloroform. An incision is then made transversely across one side of the neck with a sharp scalpel, cutting the carotid and jugular, but not the trachea or œsophagus. The blood is caught in centrifuge tubes. A similar incision may be made on the opposite side of the neck after the blood ceases to flow from the first incision. As soon as this blood clots it is ready to be centrifuged for the recovery of the serum.

Complement from different guinea pigs varies somewhat in activity. It is also very sensitive to external influences. Wall states that complement may be depended on to keep for three days, and sometimes longer. In our opinion, it cannot be relied on more than twenty-four hours after it has been drawn. Complement twelve to fifteen hours old has, however, been found to be more active than either freshly drawn samples or samples older than this. It is, therefore, advisable to draw the blood on the evening of the day before the complement is required for use and place it in the refrigerator over night. The titre is determined the following morning as per Table I.

*The Hemolysin.* The power which the blood serum of an animal of one species acquires to dissolve the red blood corpuscles of an animal of another species when injected with such corpuscles, has been known for some time. In the process of dissolution, the hemoglobin or red coloring matter of the corpuscles is liberated. The process is known as hemolysis, while the substances which effect the solution of the corpuscles are called hemolysins.

For the production of hemolysin in this work, a rabbit is immunized against horse corpuscles. The procedure to be followed in procuring and washing the horse-blood corpuscles is fully described on page 232.

We cannot express the necessity of properly washing the corpuscles better than Bureau of Animal Industry Bulletin 136,<sup>14</sup>

<sup>14</sup> Mohler, J. R. and Eichorn, A. The Diagnosis of Glanders by Complement Fixation. 1911.

which has the following to say in this regard: "The washing of the blood corpuscles must be thoroughly carried out, inasmuch as the presence of even traces of serum adhering to the corpuscles may cause difficulty in obtaining satisfactory results. If rabbits were injected with red blood corpuscles containing a small quantity of serum, the rabbits would develop, not only antibodies, or immune bodies, but also coagulins and anticomplements, and the presence of these substances would give rise to difficulties in demonstrating the presence or absence of a complete hemolysis. Furthermore, if blood corpuscles containing even traces of serum were used in the tests, it might produce a fixation of the complement, and thereby give rise to errors. Such errors would occur particularly if the hemolytic action of the rabbit serum was not very high."

Equal parts of the washed corpuscles and salt solution, heated to the body temperature ( $37.5^{\circ}$  C.), are mixed. Fourteen cubic centimeters of this suspension are injected intraperitoneally. At the end of seven days 20 c. c. are similarly administered, and after another seven days 24 c. c. more.

From six to ten days after the last injection a sample of blood is taken from an ear vein of the rabbit for examination. This blood serum should possess the power of dissolving the red blood corpuscles of the horse. In other words, it should be hemolytic for horse corpuscles.

The titre of this rabbit serum or hemolysin, as it is now termed, is next established according to Table II. If it is found to possess sufficient hemolytic power for use, the rabbit is subjected to a further bleeding. One method is to draw the blood from the ear veins into test tubes which will fit the centrifuge. The rabbit is not killed in this instance and may be used later to furnish serum by additional injections with horse corpuscles. One subsequent injection will usually stimulate a further production of hemolytic bodies. In case the blood does not flow freely from the severed vein, the application to the base of the ear, of a pledget of absorbent cotton saturated with hot water will be found useful to bring about an engorgement of the vessels. It appears almost needless to mention the necessity of shaving and disinfecting the ear prior to the bleeding.

The other method requires the destruction of the animal. It is chloroformed and the thoracic cavity is opened under aseptic

conditions. The heart is punctured to allow the blood to escape into the mediastinum, from which it is at once drawn by sterile pipettes and placed in test tubes to be centrifuged.

The serum is now recovered and preserved. Here again, either of two methods may be followed. The first consists of drawing the serum into small sealed tubes with a capacity of about 2 c. c. each. No preservative is added, so strict observance of the rules governing asepsis is necessary. In the second method the hemolytic serum is placed in 10 c. c. test tubes and mixed with 10% of the phenol-glycerin-salt solution. Later it may be drawn into the smaller tubes and sealed.

Inactivation, the next step, is carried out by heating in a water bath at 56° C. for a half hour. The hemolysin is stored in the icebox where it will keep for some weeks. The titre should be re-established every two weeks or thereabout, as the serum in some cases has been found to lose its activity quite rapidly after storage.

In order to avoid unpleasant interference in hemolysin production it is well to start two or three rabbits at the same time. Occasionally a rabbit is not adapted to the production of hemolytic substances; in other cases death may result from the injections or other causes.

At one time it was thought that the blood serum of a certain species of animals, after they had been injected with the erythrocytes of another species would hemolyze the erythrocytes of all members of this last species and of some, but not all, other species. We have found that this does not always hold true, at least in so far as horses are concerned. Ehrlich, Morgenroth and others in their experiments with goats were able to demonstrate a difference in similar cells of the same species.

We have shown that the hemolysins stimulated in a rabbit by the injection of the red blood cells of a certain horse are not specific for all horses. In a series of tests in which the red blood corpuscles from a number of different horses were used with a hemolysin produced by injecting a rabbit with red cells from a certain horse, approximately 50% resulted in complete hemolysis. In the balance the reaction was partially or wholly inhibited, showing that not all horses furnish red cells of uniform character. For this reason the same horse should be used throughout this work. Otherwise, unreliable results might be

obtained that would invalidate all tests in which such corpuscles were employed.

*The Washed Red Blood Horse Corpuscles.* The red blood corpuscles of a horse are employed in the preparation of the hemolysin, also as an essential factor in the test proper. The horse has been found to be the most suitable animal from which to obtain blood because no restraint, other than a twitch, is necessary when small quantities are drawn. The goat or sheep may be employed for this purpose if found desirable.

The jugular vein is tapped in a manner similar to that already described with cattle. The place for the insertion of the needle should be disinfected with a pledget of absorbent cotton moistened with alcohol. A bottle containing small glass beads for defibrination of the blood is provided to catch such quantity as required. The blood is defibrinated by shaking the bottle for a few minutes, after which it is filtered through thin layers of absorbent cotton or sterile gauze into test tubes of about 10 c. c. capacity. These are placed in a centrifuge, with a speed of 2000-3000 revolutions per minute, until all the corpuscles are thrown down. The supernatant serum is pipetted off, and a corresponding amount of sterile physiological salt solution added to remove the remaining traces of serum. This washing should be repeated four times, in order to dispose of all the serum, a very necessary procedure.

#### TITRATION OF THE COMPLEMENT

In the table and figure illustrating the titration of the complement, as well as in those for the other components, actual results as *usually* obtained in practice are given, in order to give the reader a clear conception of the reaction. The various quantities of the fluids thus determined are later brought together in the test of the suspected animal's serum. It must be understood that these results are those most frequently seen in practice; however, decided variations sometimes occur.

As has been stated, the complement is derived from the blood of guinea pigs. We aim to establish the smallest amount which, with a definite quantity of hemolysin, will induce a complete hemolysis of 0.5 c. c. of a 1% suspension of the horse-blood cells in salt solution. Care must be observed in all these titrations that the exact amounts of the different fluids are used, and that





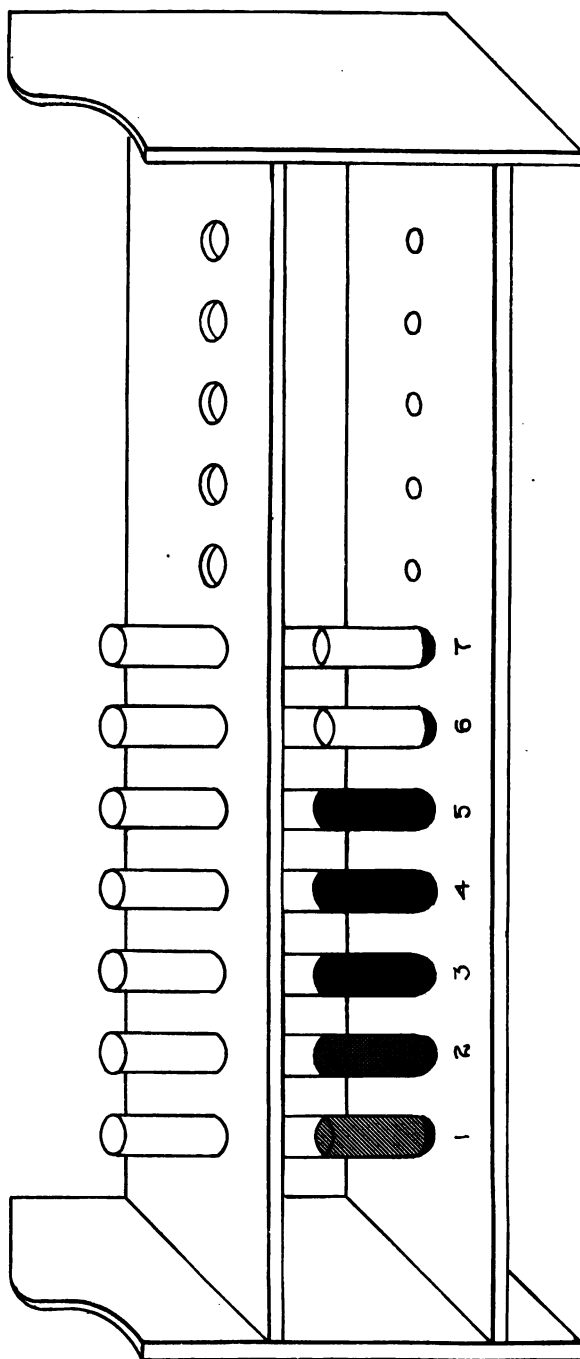


FIGURE 3. TITRATION OF THE COMPLEMENT  
Example of results usually obtained.

the dilutions are made according to directions. Carelessness will lead to results entirely at variance with actual facts. The pipettes required in the test are of three sizes, viz., 0.1 c. c. graduated to 0.01 and 0.001, 1.0 c. c. graduated to 0.1 and 0.01, and 5.0 c. c. graduated to 0.1. All glassware should be carefully cleansed. Rinsing all tubes and pipettes in distilled water just before use is recommended by the most successful technicians. Five tenths c. c. of the complement mixed with 2 c. c. of the salt solution is a sufficient amount of the complement for this titration. Two tenths c. c. of the diluted hemolysin is assumed to represent the quantity of this substance required. Seven test tubes of about 6 c. c. capacity are arranged in a rack and into each are carefully measured different amounts of the necessary components as per Table I.

TABLE I. TITRATION OF THE COMPLEMENT

Tube	1	2	3	4	5	6	7
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
NaCl solution <i>a</i> .....	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Hemolysin <i>b</i> .....	0.2	0.2	0.2	0.2	0.2	0.2	0.0
Suspension blood corpuscles <i>c</i> .....	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Complement <i>d</i> .....	0.02	0.04	0.06	0.08	0.1	0.0	0.1
Result after 1½ hours <i>e</i> .....	*	*	+	+	+	—	—

*a* 0.9% sodium chloride solution.

*b* 1% dilution hemolysin in sodium chloride solution.

*c* 1% suspension washed horse-blood corpuscles in sodium chloride solution.

*d* 20% solution complement in sodium chloride solution (0.5 c.c. complement to 2 c.c. salt solution.)

*e* + sign indicates complete hemolysis; — sign indicates no hemolysis; \* signifies a variable reaction according to the activity of the complement.

Shake each tube and place the rack in the incubator for 1½ hours, then read the results. The titre of the complement is represented by the smallest quantity which completely dissolves the red corpuscles and leaves a clear red solution. Some one of the first five tubes will contain this quantity. In the titration of the sample from which Figure 3 was taken, tube 3 represents the titre. Tubes 6 and 7 are controls; 6 should show no hemolysis as no complement is present; 7 is a control for the guinea pig serum to ascertain that it does not contain hemolytic substances; all rabbit serum is, of course, excluded from this tube. Complement with a lower titre than 0.08, represented by tube 4, should be discarded.

In the application of the complement binding test, *one and one-half* times the titre of the complement, as found by the above titration, is used. In order to facilitate measurement in the further tests, the undiluted complement and the salt solution are mixed in such a way that each 0.1 c. c. of the dilution contains the correct quantity of complement.

#### TITRATION OF THE HEMOLYSIN

The object of this titration is to establish the proper quantity or unit necessary for use in the complement fixation test. A unit of hemolysin is the smallest quantity which will bring about the complete solution of 0.5 c. c. of a 1% suspension of horse-blood corpuscles in the presence of the proper quantity of complement. The precaution of heating fresh hemolysin to 56° C. must be observed before diluting it, in order to destroy any complement which it may contain and which would in conjunction with the hemolytic bodies be capable of stimulating hemolysis. A series of eight test tubes is arranged. Into each is measured 1.5 c. c. of salt solution, then the diluted hemolysin, followed by the suspension of horse-blood corpuscles and the solution of the complement as indicated in Table II.

TABLE II. TITRATION OF THE HEMOLYSIN

Tube	1	2	3	4	5	6	7	8
	c. c.	c. c.	c. c.	c. c.	c. c.	c. c.	c. c.	c. c.
NaCl solution <i>a</i> .....	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Hemolysin <i>b</i> .....	0.02	0.03	0.05	0.1	0.15	0.25	0.15	0.0
Suspension blood corpuscles <i>c</i> .....	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Complement <i>d</i> .....	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1
Result after 14 hours <i>e</i> .....	*	*	+	+	+	+	—	—

*a* 0.9% sodium chloride solution.

*b* 1% dilution hemolysin in sodium chloride solution.

*c* 1% suspension washed horse-blood corpuscles in sodium chloride solution.

*d* Complement of known titre diluted so that 0.1 c. c. contains 14 times the established quantity.

*e* + sign indicates complete hemolysis; — sign indicates no hemolysis; \* signifies a variable reaction according to the activity of the hemolysin.

Shake each tube well, place the rack in the incubator at 37° C. for 1½ hours, after which a reading is made (See Figure 4). The standard or titre of the hemolysin is, as already stated, the smallest quantity which completely dissolves the red blood cor-

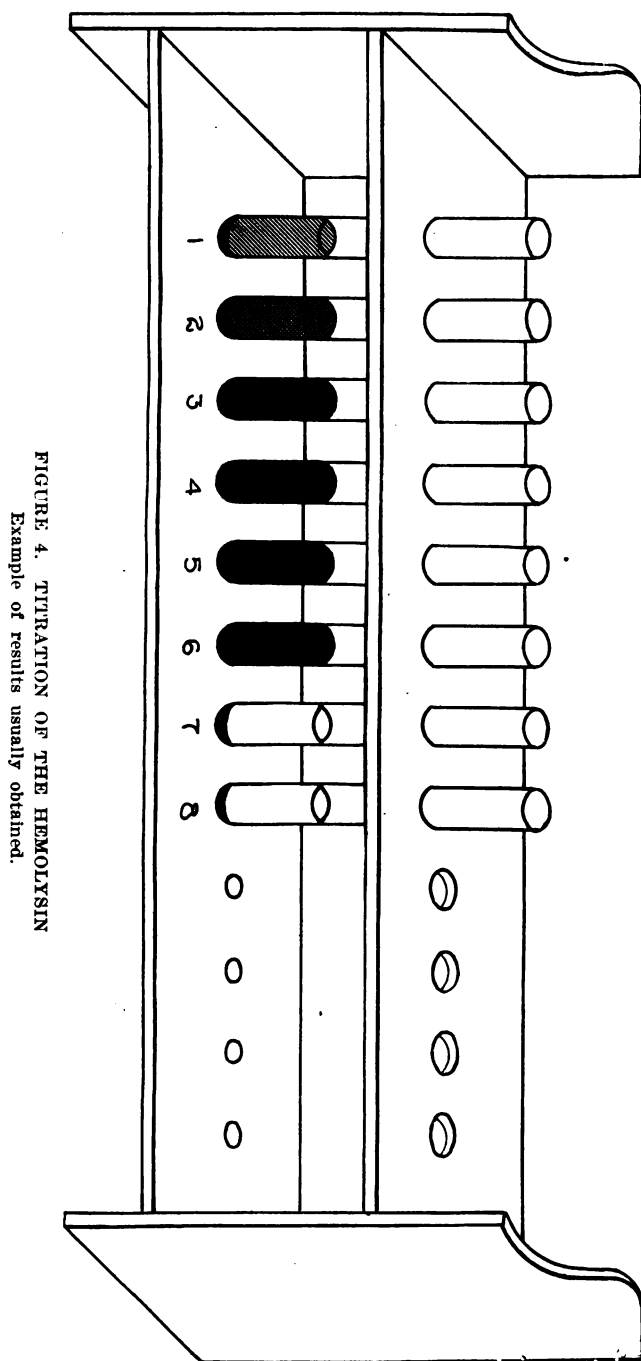


FIGURE 4. TITRATION OF THE HEMOLYSIN  
Example of results usually obtained.

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puscles and leaves a clear red solution. Hemolysis may appear in any of the first six tubes. Tube 7 is a control, with no complement, to demonstrate that the hemolysin is properly inactivated and that it alone without the addition of the complement does not have a hemolytic effect. Tube 8 is likewise a control to prove that the complement without the hemolysin will not provoke hemolysis.

The hemolysin is used in *triple* strength for all further titrations, so as to insure a sufficient quantity for the solution of the horse corpuscles. The titre of the diluted hemolysin should not be less than 0.1 which is represented in tube 4. Serum with a lower titre than this should not be used, as the larger quantities that would be required might interfere with the reaction.

#### TITRATION OF THE ANTIGEN

Our object here is to determine the smallest quantity of antigen which will prevent hemolysis when the other factors are present in the proper quantities. A series of twelve small test tubes is arranged as in the previously described titrations, and the different fluids are added as indicated in Table III. It will

TABLE III. TITRATION OF THE ANTIGEN

Tube	1	2	3	4	5	6	7	8	9	10	11	12
	c. c.	c. c.	c. c.	c. c.	c. c.	c. c.	c. c.	c. c.	c. c.	c. c.	c. c.	c. c.
NaCl solution <i>a</i> .....	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Positive cow serum <i>b</i> ....	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.0	0.0	0.0	0.0	0.0
Negative cow serum <i>c</i> ....	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.02	0.0	0.0	0.0	0.0
Antigen <i>d</i> .....	0.02	0.03	0.05	0.1	0.15	0.2	0.25	0.15	0.15	0.2	0.25	0.3
Complement <i>e</i> .....	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Incubate for 14 hours, then add the hemolytic system as below.												
Hemolysin <i>f</i> .....	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Suspension blood corpuscles <i>g</i> .....	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Result after 2 hrs <i>h</i> .....	*	*	—	—	—	—	—	+	+	+	+	*

*a* 0.9% sodium chloride solution.

*b* Inactivated cow serum known to contain the specific antibodies.

*c* Inactivated serum from a cow known to be free from abortion infection.

*d* Emulsion of a culture of *B. abortus* in carbolyzed salt solution.

*e* Complement of known titre diluted so that 0.1 c. c. contains 14 times the established quantity.

*f* Hemolysin of known titre diluted so that 0.2 c. c. contains three times the established quantity.

*g* 1% suspension washed horse-blood corpuscles in sodium chloride solution.

*h* + sign indicates complete hemolysis; — sign indicates no hemolysis; \* signifies a variable reaction according to the activity of the antigen.

be observed that an incubation is required to bring about the desired reaction before the hemolytic system is added.

After a further incubation of two hours the results are read according to the degree of hemolysis. (See Figure 5). Some one of the first seven tubes will indicate the titre, i. e., the first one of the series which shows no hemolysis. In the illustration, tube 3 containing 0.05 c. c. represents the titre. It is always required that the antigen be strong enough to bind the complement in tube 3 containing 0.05 c. c. together with 0.02 c. c. serum from a known positive reactor, an antigen of lower titre should not be used. Tube 8 with the negative serum should always show complete hemolysis. Tubes 9, 10, 11, and 12 are to demonstrate that the antigen in each, without the presence of a serum containing the specific antibodies will not prevent hemolysis. Tube 12 with 0.3 c. c. antigen ordinarily should not fix the complement.

The quantity of the antigen as determined by this titration is not taken for further work; instead, *four times* this quantity is used, as will be noted in Table IV.

#### METHOD OF PERFORMING THE COMPLEMENT FIXATION TEST

All animals infected with the abortion bacillus develop certain specific anti-bodies or immune bodies in their blood. These bodies will vary in quantity and quality depending upon the individual animal as well as the character of the infection. For this reason it is necessary to use different quantities of the suspect's serum in the final test. As exact amounts are required, special care is necessary in measuring them.

It is understood that the previously described titrations have been carried out and the titre or standard of each component established. All the substances should have a high titre. A rack with eight small test tubes is arranged for each animal to be tested. Measure the required amounts of salt solution, serum, antigen, and complement into the respective tubes, shake each tube well, and incubate for one and one-quarter hours. Then add the hemolytic system according to Table IV.

After the test tubes have been incubated again for two hours the results should be read. (See Figures 6 and 7). The degree of hemolysis is the indicator. The first four tubes are controls. Tube 1 containing salt solution and corpuscles only,

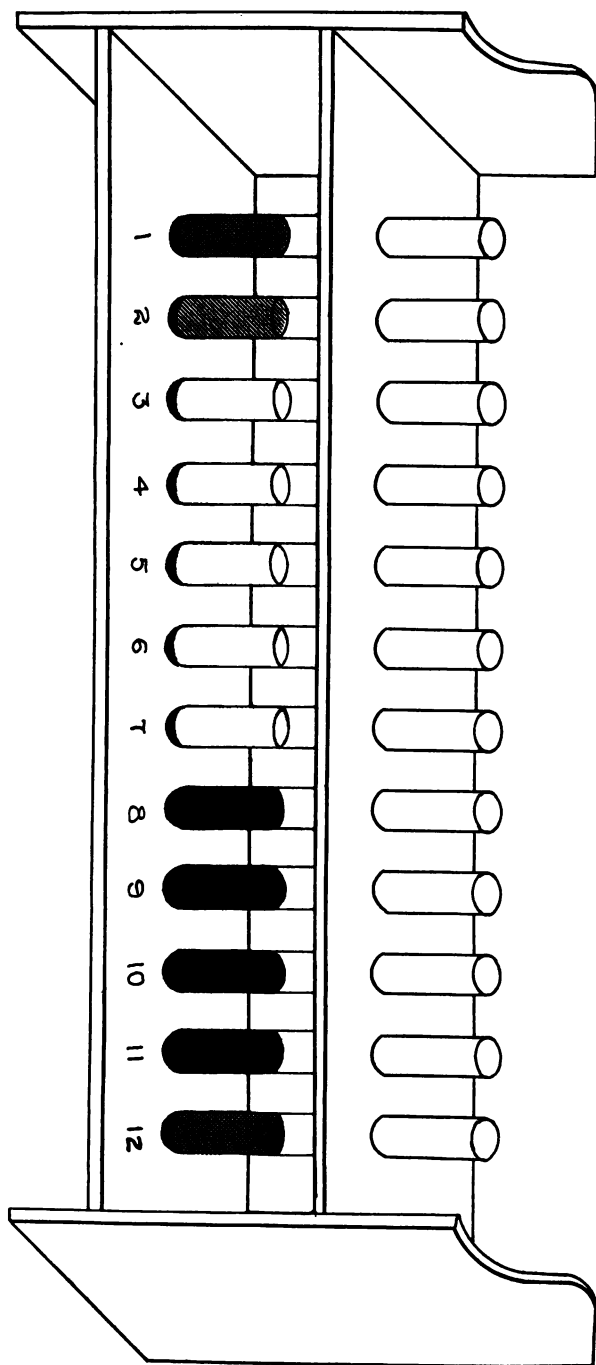


FIGURE 5. TITRATION OF THE ANTIGEN  
Example of results usually obtained.





should show no hemolysis. Hemolysis should always occur in tube 2, as the complement and hemolysin are present in the proper quantities to bring about the solution of the horse corpuscles. Tube 3 should likewise show complete hemolysis because there is no antibody present to bind the complement; further, this tube demonstrates that the antigen in the quantity used throughout the test will not of itself bind the complement. Tube 4 is to show that the antigen will not stimulate hemolysis

TABLE IV. TEST OF THE SUSPECT'S SERUM

Tube	1	2	3	4	5	6	7	8
	c. c.	c. c.	c. c.	c. c.	c. c.	c. c.	c. c.	c. c.
NaCl solution <i>a</i> .....	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Suspect's serum <i>b</i> .....	0.0	0.0	0.0	0.0	0.02	0.01	0.02	0.04
Antigen <i>c</i> .....	0.0	0.0	0.2	0.2	0.2	0.2	0.2	0.2
Complement <i>d</i> .....	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.1

Incubate for 1½ hours, then add the hemolytic system as below.

Hemolysin <i>e</i> .....	0.0	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Suspension blood corpuscles <i>f</i> .....	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Result after 2 hours <i>g</i> .....	—	+	+	—	*	*	*	*

*a* 0.9% sodium chloride solution.

*b* Undiluted cattle serum previously inactivated for 30 minutes at 56° C.

*c* Four times the amount established at the antigen titration.

*d* Complement of known titre diluted so that 0.1 c. c. contains 1½ times the established quantity.

*e* Hemolysin of known titre diluted so that 0.2 c. c. contains three times the established quantity.

*f* 1% suspension washed horse blood corpuscles in sodium chloride solution.

*g* + sign indicates complete hemolysis; — sign indicates no hemolysis; \* signifies a variable reaction according to the nature of the serum.

in the absence of the complement. Tubes 5 and 7 contain the unknown serum and are alike. They should show hemolysis if the animal is not infected with the abortion bacilli, but if immune bodies are present in the serum these tubes will both remain unchanged, indicating a complement binding. Tube 6 with a smaller amount of the suspect's serum may show binding if the immune bodies are present in sufficient quantities; a very active serum will do this. Tube 8 contains all the components with an excess of the suspect's serum; no hemolysis will take place if the animal is diseased, hemolysis if no immune bodies are present.

It must be noted that occasionally the results are not absolute, that is, there may be a partial or incomplete hemolysis in tubes 5 and 7. This is taken as an indication that the antibodies are not present in sufficient quantity to bind the complement. In such a case it is generally assumed that the animal in question has become infected recently, or that she is just recovering from an attack of the disease. In either instance a retest must be made in four to six weeks to determine positively which condition really exists. Tube 8 with the double quantity of the suspect's serum may be taken as a fairly reliable guide in these doubtful reactions.

A negative reaction does not necessarily exclude the presence of the abortion bacilli in the animal's body. The infection may have occurred so recently that the immune bodies have not been formed in sufficient quantities to bring about the reaction.

In some cases a positive reaction is obtained from the serum of a pregnant cow or heifer. This does not always mean that the animal will abort, for it has been shown that abortion is simply incidental to the infection. Experimental animals Nos. 1 and 6 (pages 240 and 241) are two cases which illustrate this point very nicely.

Occasionally anticomplements will form in an otherwise naturally negative serum after standing some time. It is therefore always best to heat old serum over the water bath for 20 minutes at  $56^{\circ}$  C. to destroy the anticomplemental substances; otherwise, in a test of such serum, what would appear like a positive reaction would in reality be negative.

Unsatisfactory results usually indicate either improperly prepared materials, or defective technique, and call for a repetition of the test. To facilitate the work when a large number of tests are to be made the same day, we advise mixing the hemolysin and the corpuscle suspension just before they are required for use in the hemolytic system.

The interpretation of the reaction may be summarized as follows:

1. Cattle in which the serum shows a complete fixation of the complement in quantities of 0.01 c. c. and 0.02 c. c. are or have been infected with abortion bacilli.
2. Cattle in which the serum gives a complete complement fixation in the quantity of 0.02 c. c. and an incomplete fixation





in the 0.01 c. c. amount, also are or have been infected with the abortion bacilli.

3. Cattle in which 0.01 c. c. of the serum shows no binding while the larger quantity gives an incomplete binding should be considered questionable reactors and retested after four to six weeks.

4. Cattle in which the serum shows no power of fixing the complement in either amount should be considered free from the infection.

If contagious abortion is found in animals of a certain herd, all should be subjected to a retest within a reasonable time. Reacting cattle should be quarantined or otherwise disposed of, and the premises properly disinfected.

The accuracy of this method of diagnosis is as great as any method based upon a biological reaction. Further experimentation may perhaps suggest some modification of the present technique. Where large numbers of animals are to be tested, such changes would be acceptable if not made at the expense of accuracy. However, at present we find it quite possible to run tests on 50 or more animals a day without undue exertion.

The test affords a reliable means by which infected animals may be detected. Proper methods of isolation and control may then be instituted by which the disease can be prevented from spreading to noninfected cows and heifers. It is a qualitative and not a quantitative test and simply indicates the presence or absence of the specific immune bodies; but clinical history has so closely corroborated the test that we may assume it to be a reliable guide as to the presence or absence of the abortion bacilli.

## ADDITIONAL EXPERIMENTAL WORK

### PURPOSE OF OUR STUDIES

The experimental work undertaken by us was first directed toward establishing the reliability of the complement fixation test. After this was accomplished, and the technique of manipulation perfected, we made use of the delicate reaction to check up other experiments.

Endeavor has been made to find some practical means by which contagious abortion might be controlled. Among other

things it is of course of utmost importance to know if this new test was rigidly accurate when applied to the same animal at stated intervals; also whether environmental conditions influenced such reactions. To establish certain of the above points the following experiments have been carried out.

#### CONSECUTIVE TESTS IN THE INFECTED HERD

The herd selected as most suited for systematic testing belonged to the Experiment Station and afforded valuable experimental material, due to the fact that it was composed of heifers which had never calved and cows which were known to have aborted. There were ten animals in the "infected" herd which was under quarantine at a separate farm. The heifers had been unavoidably exposed to the infection through contact with the diseased cows in January 1911. A short history of each animal follows:

#### HISTORY OF ANIMALS IN THE INFECTED HERD

Heifer 1 (Cora) dropped a calf at full term, April 29, 1911. The calf was apparently normal in every respect but died in half an hour. The placenta and fluids were caught in sterile containers and immediately taken to the laboratory for examination. Media were inoculated but no growths resembling the abortion bacilli could be found. The heifer's blood was tested by the complement fixation method in July and gave a positive reaction. For subsequent tests see Table V. This heifer has come in heat at irregular periods for more than one year and has been served, but has apparently failed to conceive, indicating a pathological condition of the genital organs due to the infection. Possibly conception took place at the first or second service and a second abortion occurred so early in gestation that it escaped the attention of the attendant.

Heifer 2, registry No. 31002, commenced to give considerable milk July 1, 1911, although she had never calved, at which time she was given the test and responded positively. On August 1, 1911, she aborted thus confirming our diagnosis. Her fetus was recovered and taken to the laboratory where cultures were made from the stomach contents. Doctor Larson<sup>15</sup> found abor-

<sup>15</sup> Dr. W. P. Larson, now with the University of Minnesota, was associated with us in this work for a few weeks in the summer of 1911. We take this opportunity to acknowledge our indebtedness to him.

tion bacilli in large numbers on the serum-agar culture medium and prepared an antigen from the organisms. This organism he later demonstrated to be identical with the Bang bacillus.

Heifer 3 (Agnes), a pure bred Holstein, aborted February 19, 1911. First test for abortion was made in July, 1911, when the reaction was positive. Further tests showed the reactions noted in Table V.

Heifer 4, an Ayrshire, aborted May 29, 1911. A peculiar feature about this heifer was the persistent negative reaction which she gave for some months after abortion, and the eventual appearance of the immune bodies as determined by the test. We are led to believe that abortion in this case was due to accidental causes.

Heifer 5, a pure bred Jersey calf, dropped March 10, 1911, about six weeks prematurely. The dam was later found to be a positive reactor. As a calf this animal sucked the infected mother and was kept in the infected herd. In spite of these excellent possibilities for acquiring the infection, the tests indicate that no antibodies could be demonstrated in her serum until she was about one year old.

Heifer 6, registry No. 31004, was dropped November 5, 1909, and served May 20, 1911. Like heifer 7 she ran with infected animals from January to October, 1911. No signs of abortion were ever noted. She was selected as a good animal to place under the carbolic acid treatment, and so was isolated with heifers 5 and 7 in October. The medication consisted of subcutaneous injections of 8 c. c. of a 3% solution of the acid every ten days. On January 30, 1912, the strength of the carbolic acid solution was increased to 5%. She calved February 19, 1912. Portions of the placenta were immediately taken to the laboratory, also some fluid from the parturient uterus; media were inoculated and the organism isolated from scrapings of the cotyledons. An antigen prepared from this growth gave a typical binding when titrated against the serum of a known positive reactor. The results of experimental injections of the recovered organism into the veins of a pregnant ewe are noted on page 244. There can, therefore, be no question that the abortion bacilli are present in this heifer. It is interesting to note that a test of the blood serum obtained from the calf when it was 25 days old gave a complete fixation of the complement. This case demon-



strates very nicely that cows may harbor the infectious agent, yet not always abort. Such animals are, however, dangerous sources of infection and may transmit the disease to others.

Heifer 7, registry No. 31001, was dropped October 27, 1909, and served about April 17, 1911. She ran with infected animals from January to October, 1911, when she was isolated with heifers 5 and 6 at which time carbolic acid treatment was inaugurated. The dose in this case was 2 c. c. of the pure acid diluted in water and placed on the bran mash every other day, night and morning. On January 30, 1912, the dosage of carbolic acid was increased to 3 c. c. Abortion occurred December 21, 1911. The fetus and fetal membranes were taken to the

TABLE V. SUMMARY OF CONSECUTIVE TESTS IN THE INFECTED HERD  
+ sign indicates positive reaction; — sign indicates negative reaction; ? mark denotes atypical reaction.

No.	1911 July test	1911 Oct. test	1911 • Dec. test	1912 Jan. test	1912 Mar. test	1912 Apr. test	Abortion history
1....	+	+	.....	+	+	.....	Calved 4/29/11
2....	+	+	+	+	+	+	Aborted 8/1/11
3....	+	+	+	+	+	+	Aborted 2/19/11
4....	—	—	?	+	+	+	Aborted 5/29/11
5....	—	—	—	—	?	—	Dropped 3/10/11
6....	—	?	?	+	+	+	Calved 2/19/12
7....	—	?	+	+	+	+	Aborted 12/21/11
8....	+	+	+	+	+	+	Probably aborted 8/11
9....	+	—	+	+	.....	.....	Aborted years ago
10....	.....	—	—	+	+	.....	Bull

laboratory and different media inoculated from the stomach contents, heart's blood, and with scrapings from the placental cotyledons. Cultures of the abortion bacillus were obtained on the serum-agar plates from the stomach contents of the fetus. These were eventually grown in pure culture and used for the preparation of antigen which proved to be specific when titrated against serum from known positive reactors, thus establishing the identity of the organism.

Heifer 8, registry No. 31003, was dropped November 4, 1909, and bred in May, 1911. While at pasture in August, 1911, she was seen to have a slight vaginal discharge which was attributed to œstrum so no attention was given the animal. Recurrence of œstrum was noted in October and again in December. A positive reaction was given with her serum in July so it seems likely that this heifer aborted at pasture. This belief is sub-

stantiated by recurrence of oestrus and the fact that no signs of pregnancy have since been visible.

Cow 9, an aged grade Hereford, aborted some years ago (exact date not known). This cow had retained placenta, and milk fever November 17, 1910, but calved normally next season.

Bull 10, a pure bred Guernsey, used exclusively for service in the infected herd.

The dates of and reactions obtained from the consecutive tests on these animals are given in Table V.

#### DISCUSSION OF RESULTS WITH CONSECUTIVE TESTS

Among the interesting points brought out by this experiment are the following:

(1) The persistence of the immune bodies longer than a year after abortion occurred, e. g., No. 3.

(2) The gradual appearance of these bodies in different cases, e. g., Nos. 4, 6, 7, 10.

(3) The fact that No. 5, a calf, did not show the immune bodies up to a year old, although she was dropped prematurely, and was in constant association with infected animals.

(4) The accuracy with which the test detected infected animals, (a) even before any clinical signs of abortion were noted, e. g., Nos. 6 and 7; (b) in the case of one, No. 8, which was supposed to be safely pregnant, but which had probably aborted.

(5) The reaction given by the herd bull, No. 10, after association with infected cows.

#### LABORATORY EXPERIMENTS

Twenty placental membranes and fetuses have been examined bacteriologically. The abortion organism was isolated from thirteen of the specimens. Some of the others were accidental abortions, as was conclusively demonstrated by subsequently testing the cows' blood serum. Microscopical identification alone has not been found reliable.

In order to further demonstrate that the organisms recovered were the cause of abortion a number of rabbits were injected intraperitoneally and intravenously with emulsions of different strains. These does aborted within a few hours after the injections which accounts for the negative results obtained from

the cultural examinations of the stomach contents and heart's blood of the fetuses, and the uterine contents of the does.

An especially interesting experiment was the one carried out on a pregnant ewe as follows:

Ewe 769 was bred November 24, 1911. On March 17, 1912, thirty-five days before she was due to lamb, she received by intrajugular injection 4 c. c. of an emulsion of abortion bacilli recovered from heifer 6. This emulsion was made by washing the growth from a 10-day serum-agar slope with 20 c. c. of normal salt solution, and was rich in abortion bacilli. On April 2, seventeen days after the injection, the ewe aborted twin lambs. One never breathed, as indicated by lung tissue which was liver-like in appearance and sank in water, showing that the alveoli had never been filled with air. The other lived for a few hours but was very weak and undersized. Plates were inoculated from the umbilicus and stomach contents of both fetuses, also from a cotyledon of the placenta. Stained material from the stomach contents revealed a small organism which appeared like the abortion bacillus. Typical colonies were obtained on the plates which had been seeded from the stomach and umbilicus, thus satisfying Koch's postulates. The ewe's blood serum was tested one week after abortion occurred and gave a decided complement fixation, indicating the presence of a large quantity of the specific immune bodies.

#### RESISTANCE OF ABORTION BACILLI

*B. abortus* seems to have considerable resistance and will live for some time outside the animal body. Bang and Stribolt were able to isolate the organism in pure culture from two fetuses which had been dead five months and nine months, respectively. The uterine exudate kept on ice contained living organisms after seven months.

Freezing does not kill this organism. On February 13, 1912, an aborted fetus was forwarded from Columbus, Wis. Upon arrival at the laboratory the calf was frozen solidly; however, the abortion bacilli were isolated by the plate method. On February 29 the fetal membranes of an aborted fetus were forwarded from Beloit, Wis. The membranes were frozen as hard as a cake of ice; nevertheless, upon cultural examination the abortion bacilli were found alive and vigorous. The organisms were recov-

ered in pure culture. Later, antigens were prepared with both strains and gave a firm binding, when titrated against a positive serum.

#### OTHER METHODS FOR THE PREPARATION OF ANTIGEN

Besides the method previously described for the preparation of the abortion antigen, the following may be mentioned:

A. To 200 c. c. sterile bouillon in a specially blown flask are added 50 c. c. sterile raw blood serum. After two or three days incubation to determine sterility, it is inoculated with abortion bacilli. Oxygen is allowed to bubble through the liquid to drive off at least 90% of the air; then it is incubated 10 to 12 days, after which it is carbolized and titrated as previously described.

B. Serum bouillon containing the growth of abortion bacilli, prepared as above, is centrifuged. The supernatant liquid is then pipetted off, leaving the organisms clumped together in the bottom of the tube. Physiological salt solution is added to two-thirds of the original volume. This suspension of abortion bacilli in salt solution is very good antigen, more satisfactory in some respects than that referred to above because the foreign horse serum has been removed.

C. Another method which has been fairly satisfactory is to centrifuge in the manner described under B. The salt solution is added, the suspension again thrown down, and the supernatant solution pipetted off. This is repeated three times until all of the serum bouillon is removed. The mass is then ground with sterile sand or pulverized glass until the organisms are broken up. Salt solution is added to one-half the original volume and the mixture placed on ice for 12 to 24 hours. The cellular elements, sand, etc. are removed by means of a porcelain filter. Antigen prepared in this manner has few advantages over that described above. It is a clear solution, and a little nicer to work with on that account, and, if properly employed, will give satisfactory results.

It was observed that some antigens, prepared by the use of serum bouillon, would bind the complement in the absence of the positive serum, in such small quantities as to make their use in the test prohibitive. It was thought that the raw horse serum in the mixture might be responsible for this. Accordingly antigens which bound the complement were treated as described un-

der C, but this, while eliminating the raw serum, did not do away with the binding. When sterile serum and bouillon are mixed and incubated there is oftentimes a copious precipitate. A quantity of this precipitate was withdrawn from a flask before inoculating with the abortion bacilli. The content of the flask was sterile as shown by cultural and microscopical examination. This precipitate bound the complement in .04 to .06 c. c. and partially in 0.02 c. c., showing that it was responsible for the unpleasant fixation noted above.

Fixation of the complement has not been observed in antigens prepared as described on page 228, in any amount up to 0.3 c. c.; consequently that method is to be recommended as the most satisfactory.

#### FIELD EXPERIMENTS

In order to show the practical value of this new diagnostic method, it was necessary to conduct tests in different herds. Concrete examples of the conditions under which the tests were made, follow:

A noted pure bred dairy herd was visited with the object of prescribing preventive and curative measures for white scours in the calves. The disease was so virulent that it had been impossible to raise calves, twelve having died within a period of two months. We had no suspicion of contagious abortion at this time, as the calves had all been carried full term, but thought it a good opportunity to try out the fixation test. Consequently samples of blood were taken from each of the six cows which had recently calved. Upon examining the blood serum it was found that, with one exception, all responded negatively and were therefore pronounced free from contagious abortion infection. The exception was a four years old cow which gave a most beautiful positive reaction. The results were reported to the owner with the request that he furnish us information as to her history. He replied that the cow did abort in 1910, as did a number of others associated with her at the time.

Another instance, which illustrates the reliability of the test even more conclusively, was a test made on some samples forwarded by Dr. L. A. Wright of Columbus, Wis. His letter under date of Feb. 10, 1912, states the facts as follows:

Your favor just at hand with record of test enclosed. I did not give you any assistance in the cases, as you know we sometimes see things

wrongly when influenced to do so by suggestions. You will be interested and pleased to know that No. 3, positive, has since aborted. No. 17 was the one which had aborted. I removed the afterbirth at once and washed her out and she has had carbolic acid every two days, in teaspoonful doses, ever since, so she is, and was at the time we sent blood samples, quite clean. No. 14 calved since we sent tubes, apparently all right, but she is an old cow and was well advanced in pregnancy before she was brought into this herd and may have harbored the germs without causing abortion. Came near forgetting to tell you that I sent you, by express, yesterday, the aborted calf of No. 13. I froze it and packed it in snow.

The calf referred to in the above letter was the one from which cultures were obtained as noted on page 244.

Mr. H. N. Longley of Dousman, Wis., writes in his letter of April 23, as follows:

Your test for abortion corresponds exactly with the history of the cases. We had little reason to suspect the disease in numbers 1 and 2. Numbers 3 and 4 are two pure breds purchased about a year ago: number 3 aborting and number 4 calving prematurely a few months after purchasing them, and both have failed to get with calf since.

These are but three of many instances which substantiate the test and which have led us to place much confidence in its accuracy.

TABLE VI. SUMMARY OF RESULTS WITH THE COMPLEMENT FIXATION  
Twenty-two infected herds tested. <sup>a</sup>

	Number of animals	Reaction					
		Positive	Negative	Questionable	Per cent positive	Per cent negative	Per cent questionable
No history of abortion.....	304	53	238	13	17.4	78.3	4.3
Known aborters.....	97	81	14	2	83.5	14.4	2.1
Herd bulls.....	10	1	9	0	10.0	90.0	0.0
Totals.....	411	135	261	15	32.9	63.5	3.6

<sup>a</sup> A very few of these animals came from herds where no infection existed, but are included for the purpose of this work.

Table VI shows the results obtained from the tests on cattle from various widely separated parts of the state. A brief analysis reveals the fact that 17.4% of the animals in infected herds which have no history of abortion, show evidence of infection with abortion bacilli. This infection may have occurred either while the animal was in utero (congenital immunity) or have been acquired after birth. These figures, of course, include a

certain number of cows that have been bought from unknown sources and which, if the actual history were known, may have aborted in the past. Only 83.5% of the cows which have been known to abort gave positive reactions. This apparent discrepancy is explained when attention is drawn to the fact that many of these animals aborted more than a year before the test was applied, while a few aborted four or five years before. It is assumed that the negative reactors in this group have lost whatever immunity they may have gained from the infection. Only one herd bull out of ten gave a positive reaction, indicating that the male in these cases rarely harbored the abortion bacilli in sufficient quantities to stimulate the formation of immune bodies in his serum. Of the entire 411 animals tested 135, or 32.9% gave positive reactions and must, therefore, be considered as harboring the abortion bacilli at the time the test was made, or at some time in the not distant past. Two hundred and sixty-one or 63.5%, were found free from the infection. Of these, fourteen had been known to abort; some, from accidental causes, others, possibly from the contagious form, but no immune bodies could be demonstrated in their sera. It must be understood that practically all these animals were in infected herds, in fact, 97 were known to have aborted; therefore, 32.9% is not a larger number of reactors than should reasonably be expected. No conclusions as to the prevalency of the disease can be gathered from these figures because the average dairy herd is not represented, instead only those herds have been included in which the infection was known to exist.

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